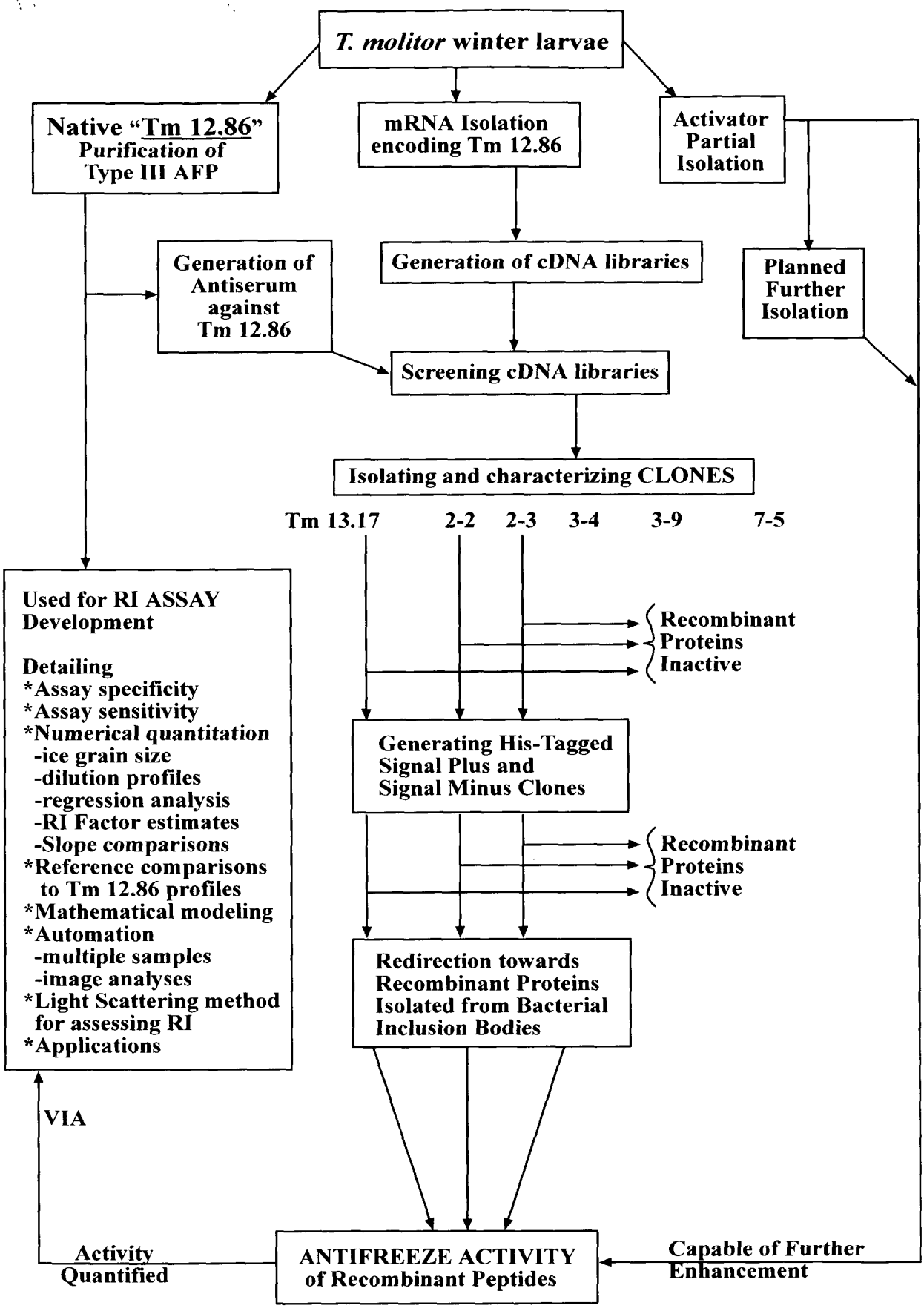
T. molitor winter larvae
Native "Tm 12.86" Purification of Type III AFP
mRNA Isolation encoding Tm 12.86
Activator Partial Isolation
Generation of Antiserum against Tm 12.86
Generation of cDNA libraries
Planned Further Isolation
Screening cDNA libraries
Isolating and characterizing CLONES
Tm 13.17
2-2
2-3
3-4
3-9
7-5
Recombinant Proteins Inactive
Used for RI ASSAY Development
Detailing
*Assay specificity
*Assay sensitivity
*Numerical quantitation
-ice grain size
-dilution profiles
-regression analysis
-RI Factor estimates
-Slope comparisons
*Reference comparisons to Tm 12.86 profiles
*Mathematical modeling
*Automation
-multiple samples
-image analyses
*Light Scattering method for assessing RI
*Applications
Generating His-Tagged Signal Plus and Signal Minus Clones
Recombinant Proteins Inactive
Redirection towards Recombinant Proteins Isolated from Bacterial Inclusion Bodies
VIA
Activity Quantified
ANTIFREEZE ACTIVITY of Recombinant Peptides
Capable of Further Enhancement

Fig. 1.0

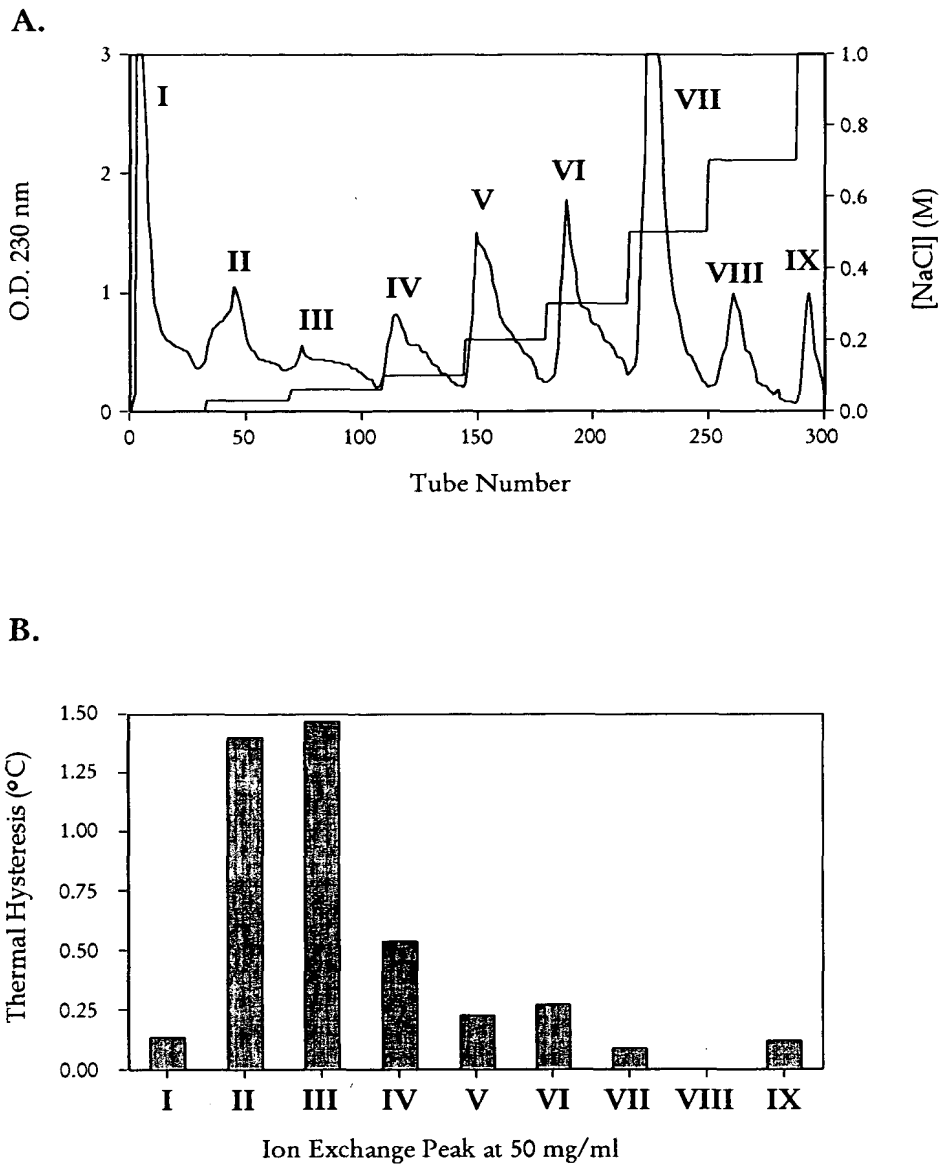
A.
O.D. 230 nm
[NaCl] (M)
Tube Number
I
II
III
IV
V
VI
VII
VIII
IX
B.
Thermal Hysteresis (°C)
Ion Exchange Peak at 50 mg/ml

Fig. 1.1

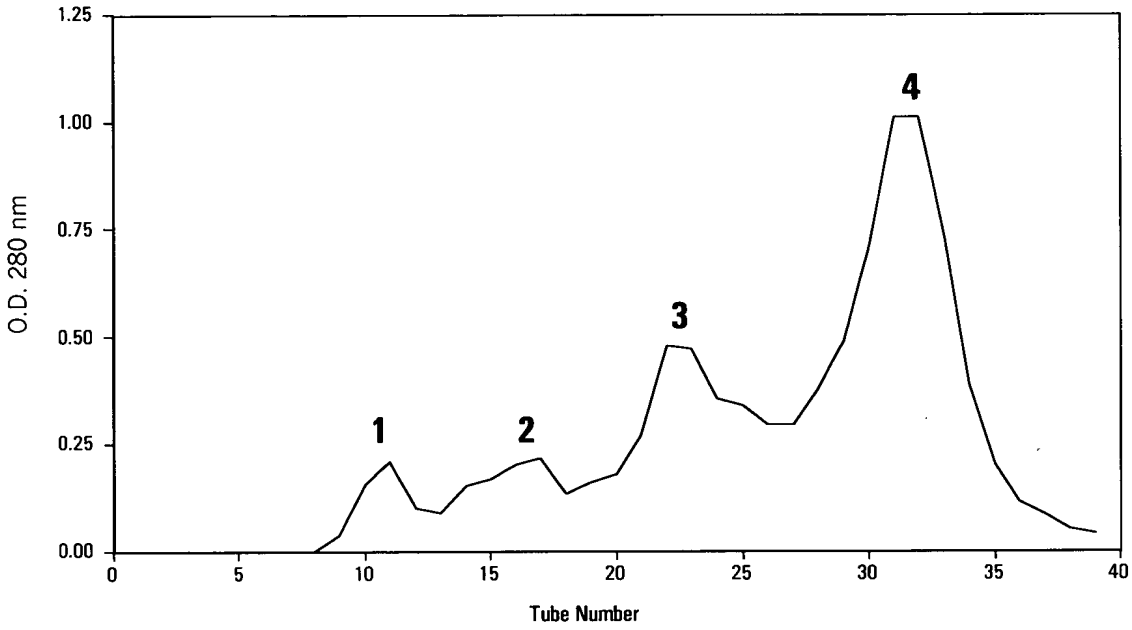
1.25
1.00
0.75
0.50
0.25
0.00
O.D. 280 nm
0
5
10
15
20
25
30
35
40
Tube Number
1
2
3
4

Fig. 1.2

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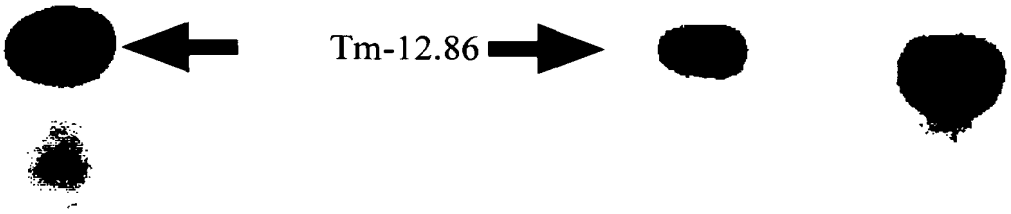


Fig. 1.3

Fig. 1.4

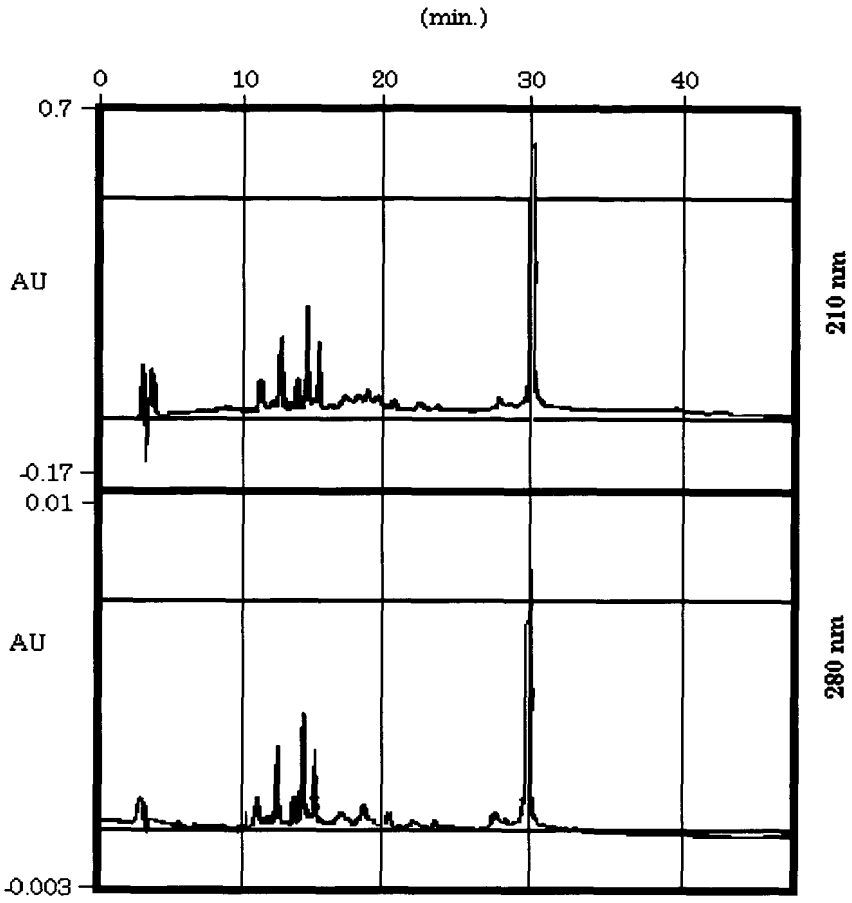


Fig. 1.5

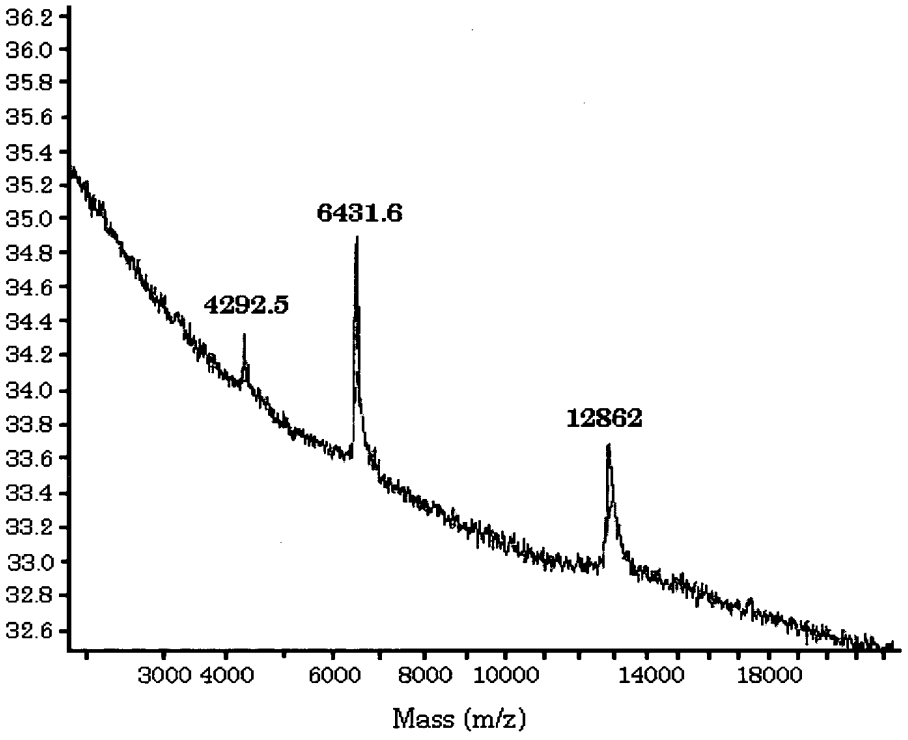

36.2
36.0
35.8
35.6
35.4
35.2
35.0
34.8
34.6
34.4
34.2
34.0
33.8
33.6
33.4
33.2
33.0
32.8
32.6
6431.6
4292.5
12862
3000 4000
6000
8000
10000
14000
18000
Mass (m/z)

Fig. 1.6

Docket No. 5050-0245

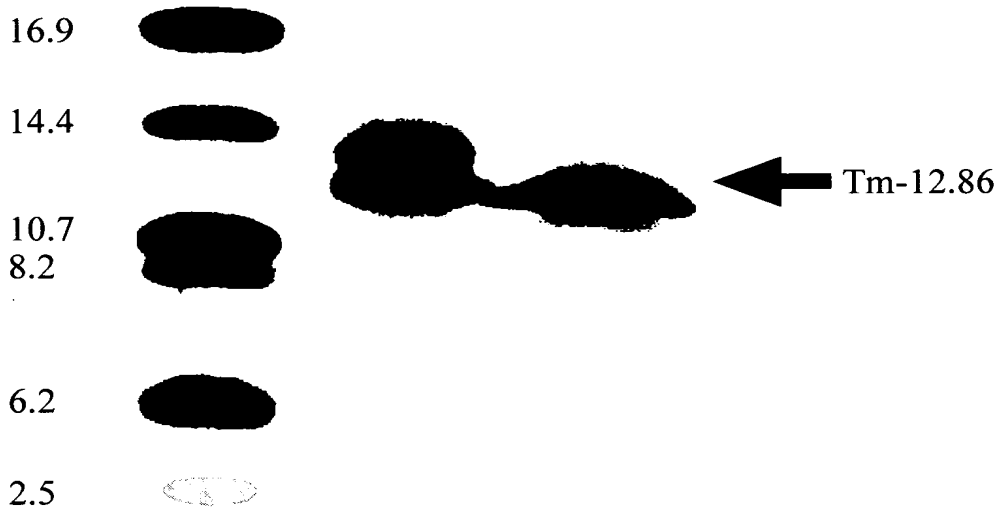


Fig. 1.7

09652365 "0E4E02

NH_2-L-T-D-E-Q-I-Q-K-R-N-K-I-S-K-E-?-Q-Q-V

Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser Lys Glu Gln Gln Val

Fig. 1.8

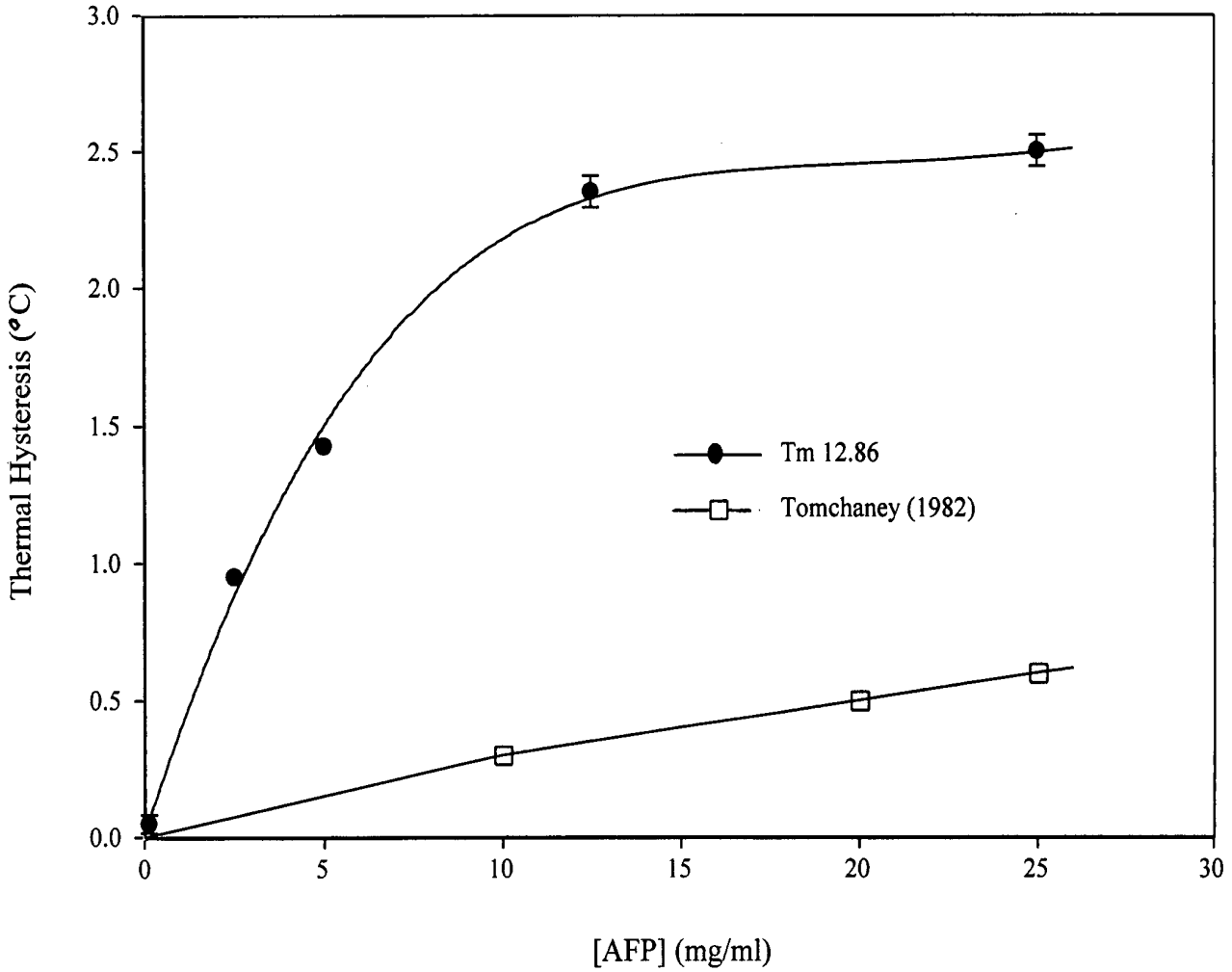

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Thermal Hysteresis (°C)
0
5
10
15
20
25
30
[AFP] (mg/ml)
Tm 12.86
Tomchaney (1982)

Fig. 1.9

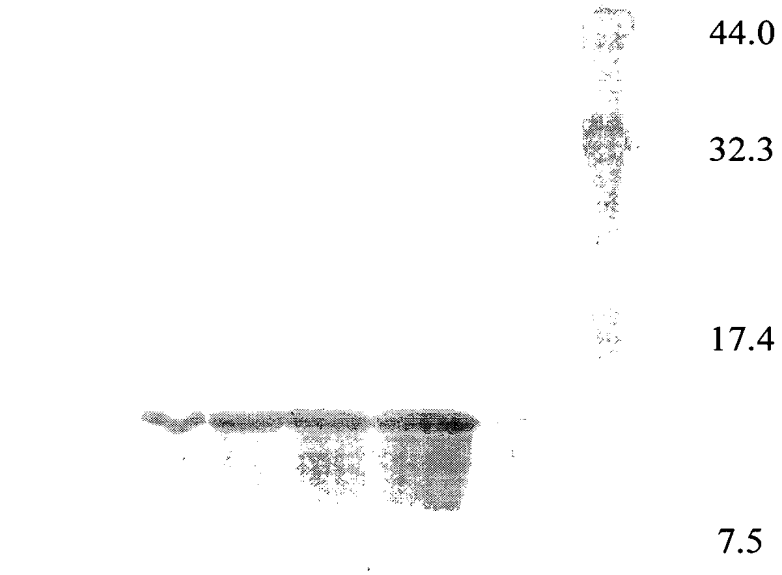


Fig. 1.10

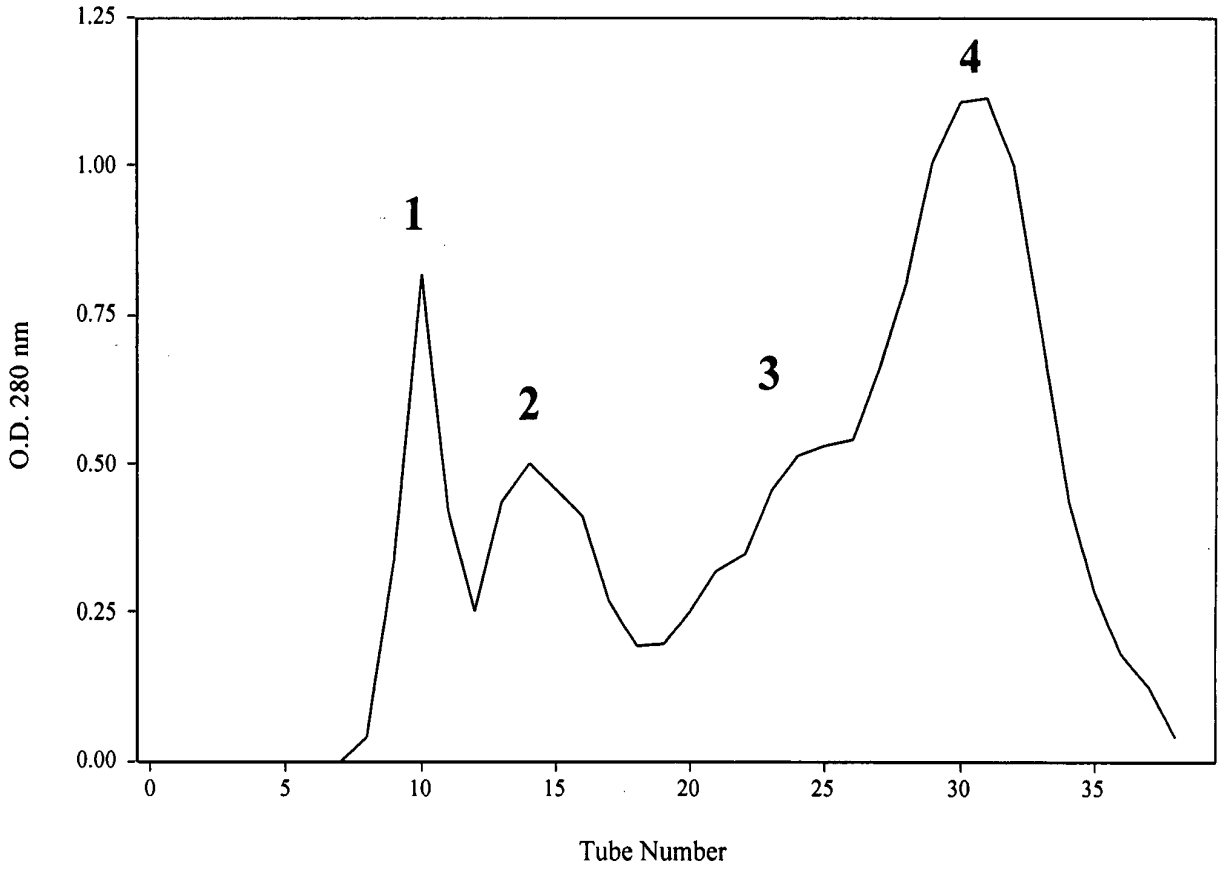

1.25
1.00
0.75
0.50
0.25
0.00
O.D. 280 nm
0
5
10
15
20
25
30
35
Tube Number
1
2
3
4

Fig. 1.11

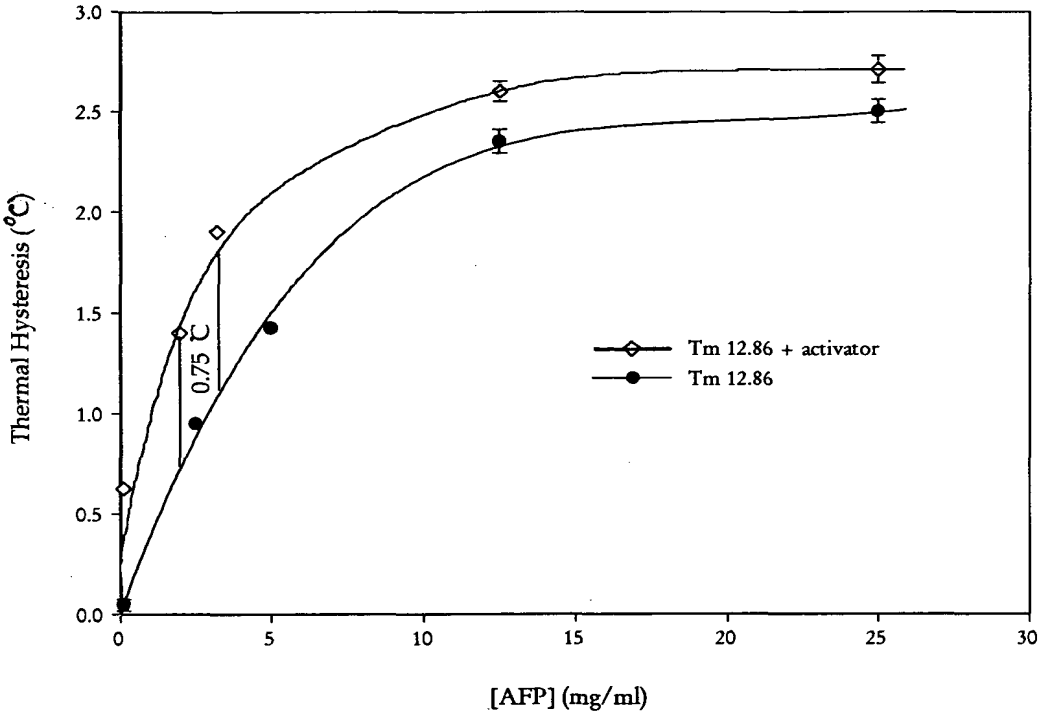

Thermal Hysteresis (°C)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
5
10
15
20
25
30
[AFP] (mg/ml)
0.75 °C
Tm 12.86 + activator
Tm 12.86

Fig. 1.12

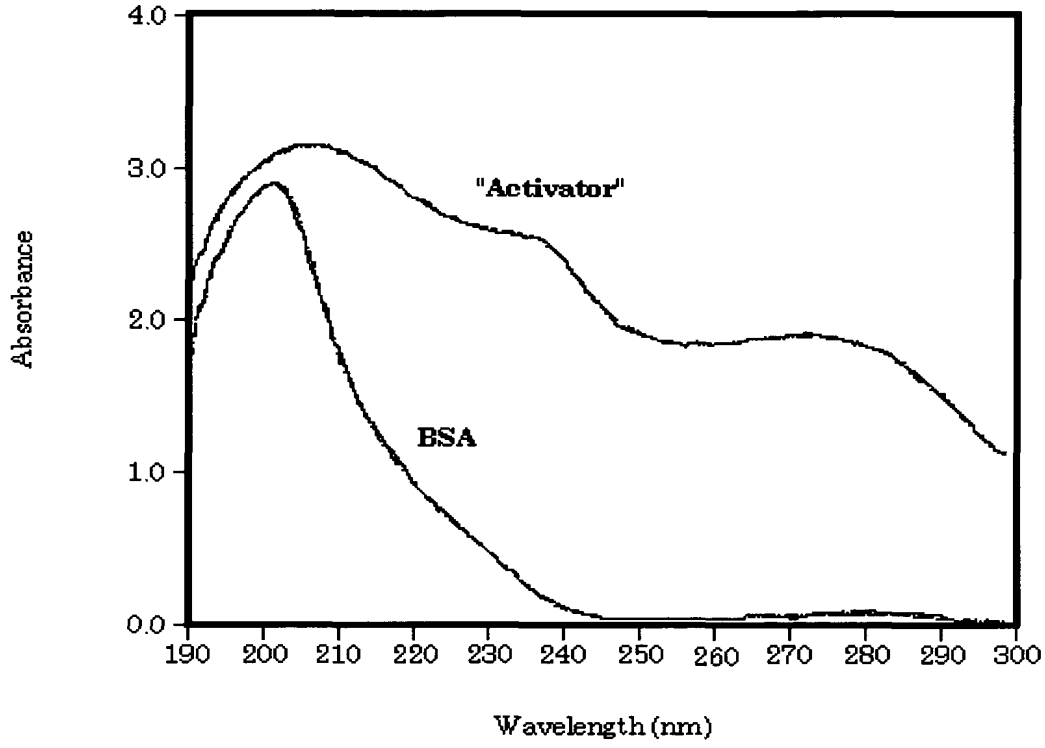

4.0
3.0
2.0
1.0
0.0
Absorbance
"Activator"
BSA
190
200
210
220
230
240
250
260
270
280
290
300
Wavelength (nm)

Fig. 1.13

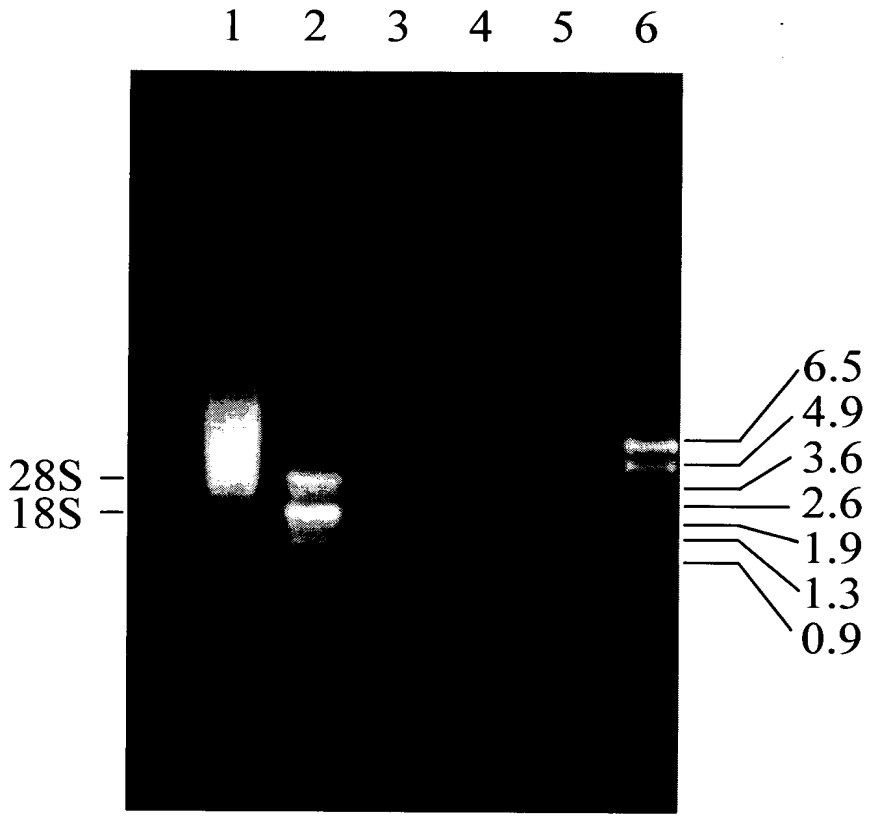


Fig. 2.0

Sep 25 05 09:57a 05-25-2005

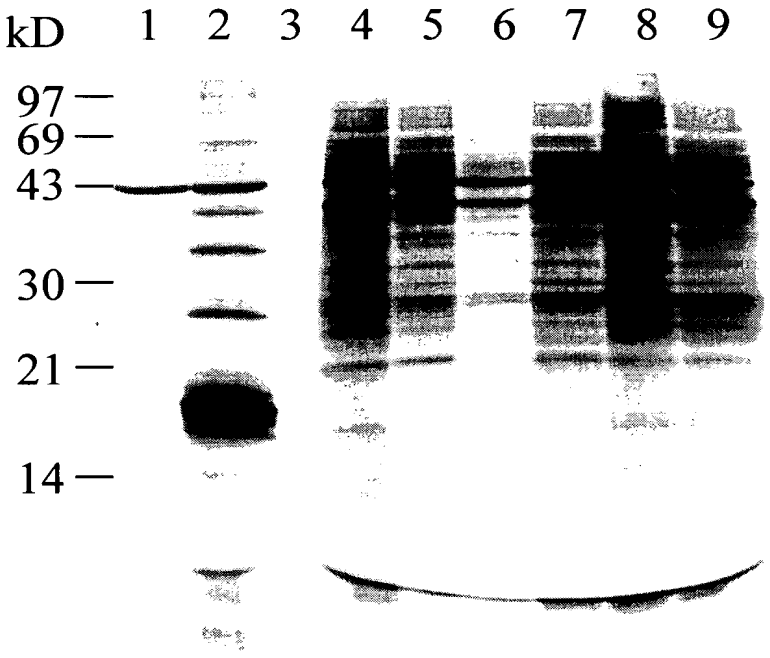


Fig. 2.1

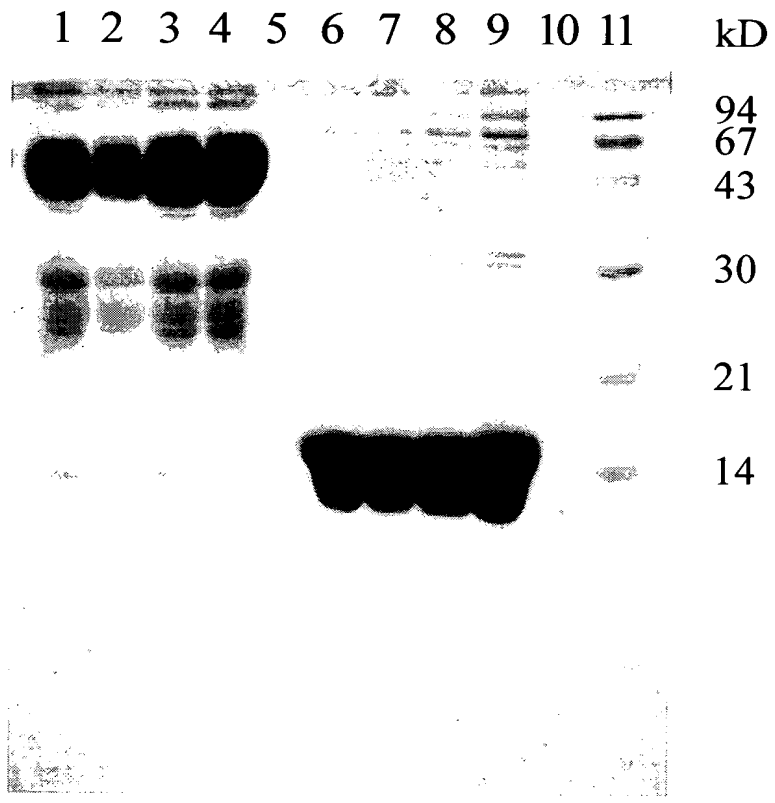


Fig. 2.2

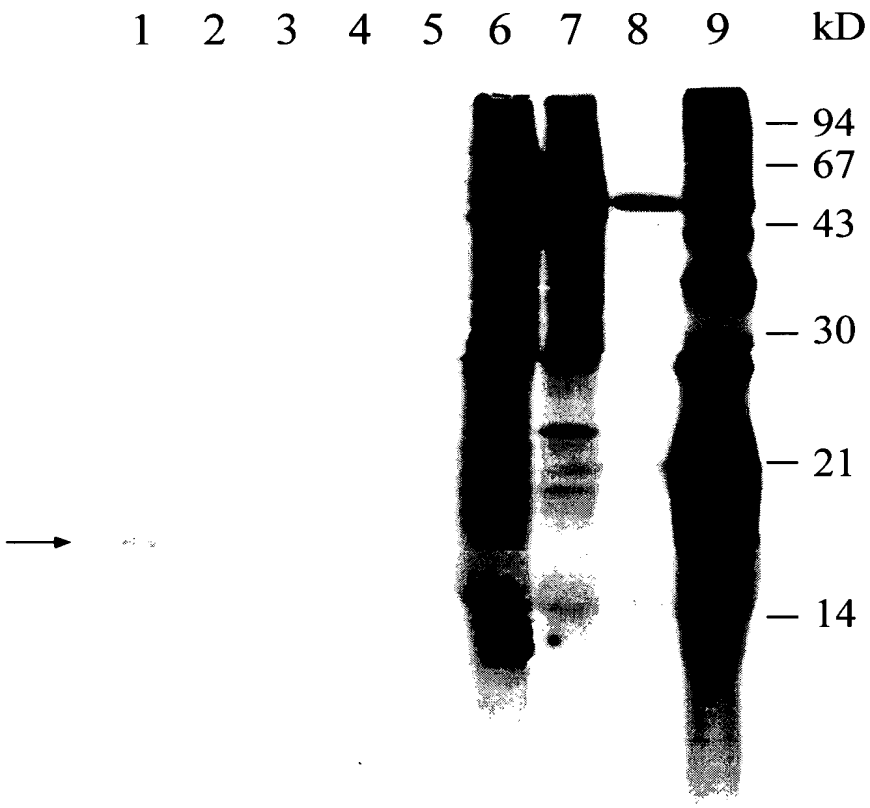
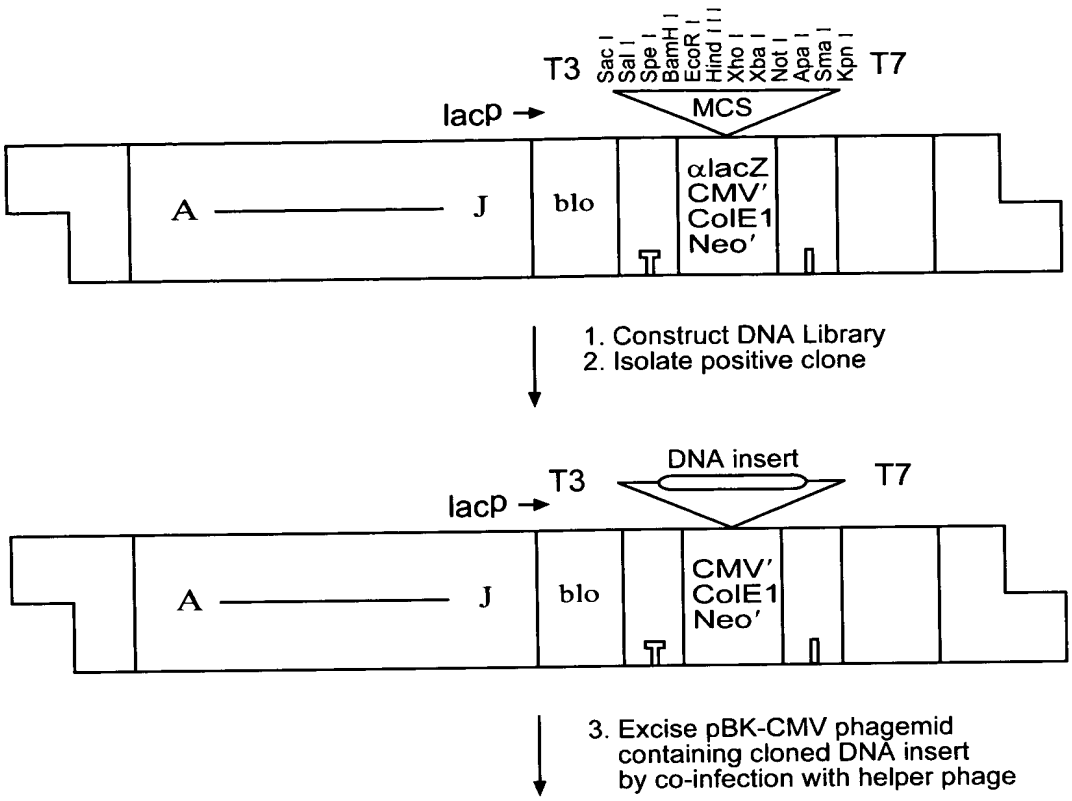


Fig. 2.3

0 9 0 3 7 0 9 6 : 0 2 4 5 0 5



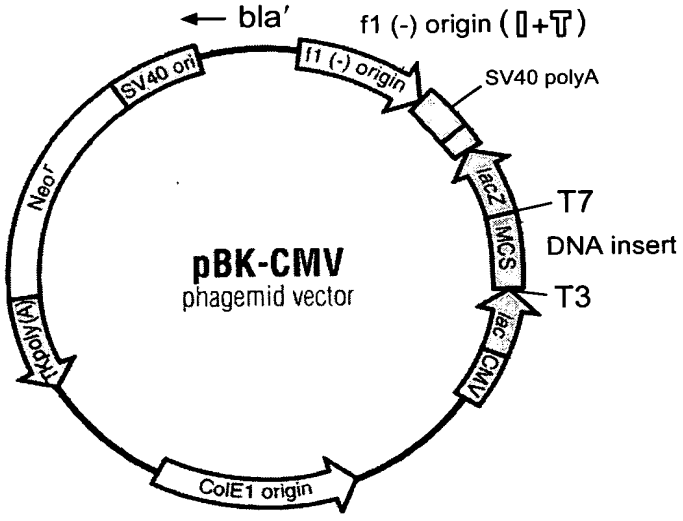
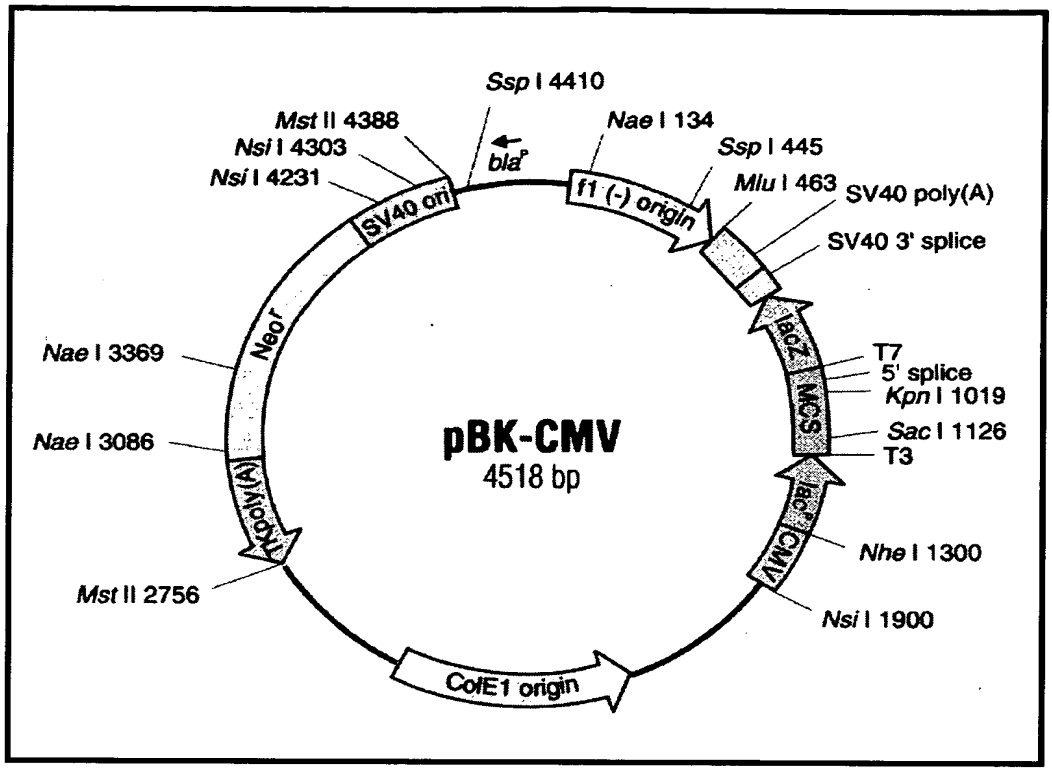


Fig. 2.4a


Ssp I 4410
Mst II 4388
Nsi I 4303
Nsi I 4231
bla^P
Nae I 134
Ssp I 445
Mlu I 463
SV40 poly(A)
SV40 3' splice
SV40 ori
f1 (-) origin
lacZ
MCS
T7
5' splice
Kpn I 1019
Sac I 1126
T3
Nae I 3369
Neo^r
Nae I 3086
pBK-CMV
4518 bp
HSV TK poly(A)
lac I
CMV
Nhe I 1300
Mst II 2756
Nsi I 1900
ColE1 origin

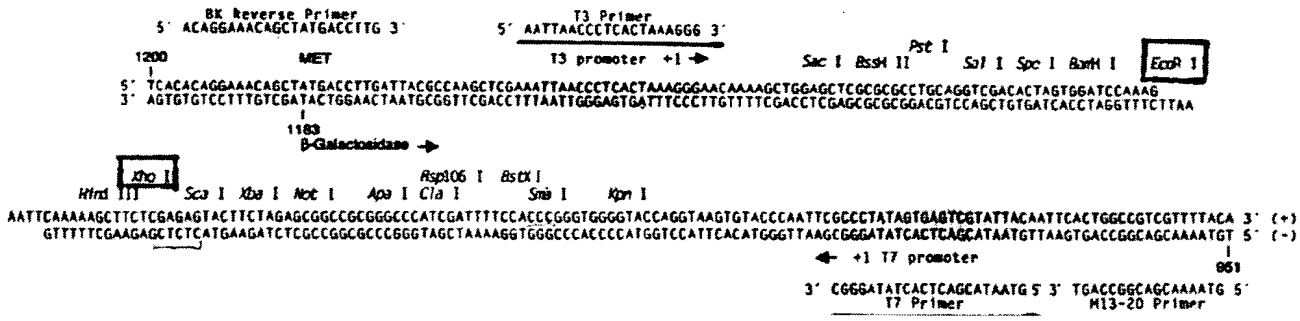

BK Reverse Primer
5' ACAGGAAACAGCTATGACCTTG 3'
T3 Primer
5' AATTAACCCTCACTAAAGGG 3'
1200
MET
T3 promoter +1
Pst I
Sac I BssH II Sal I Spe I BamH I
EcoR I
5' TCACACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCGCGCGCCTGCAGGTCGACACTAGTGGATCCAAAG
3' AGTGTGTCCTTTGTCGATACTGGAACTAATGCGGTTCGACCTTTAATTGGGAGTGATTTCCCTTGTTTTCGACCTCGAGCGCGCGGACGTCCAGCTGTGATCACCTAGGTTTCTTAA
1183
β-Galactosidase
Xho I
Hind III
Asp106 I
BstX I
Sca I Xba I Not I Apa I Cla I Sma I Kpn I
AATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGGCCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACA 3' (+)
GTTTTTCGAAGAGCTCTCATGAAGATCTCGCCGGCGCCCGGGTAGCTAAAAGGTGGGCCCACCCCATGGTCCATTCACATGGGTTAAGCGGGATATCACTCAGCATAATGTTAAGTGACCGGCAGCAAAATGT 5' (-)
+1 T7 promoter
951
3' CGGGATATCACTCAGCATAATG 5' 3' TGACCGGCAGCAAAATG 5'
T7 Primer
M13-20 Primer

Fig 2.4b

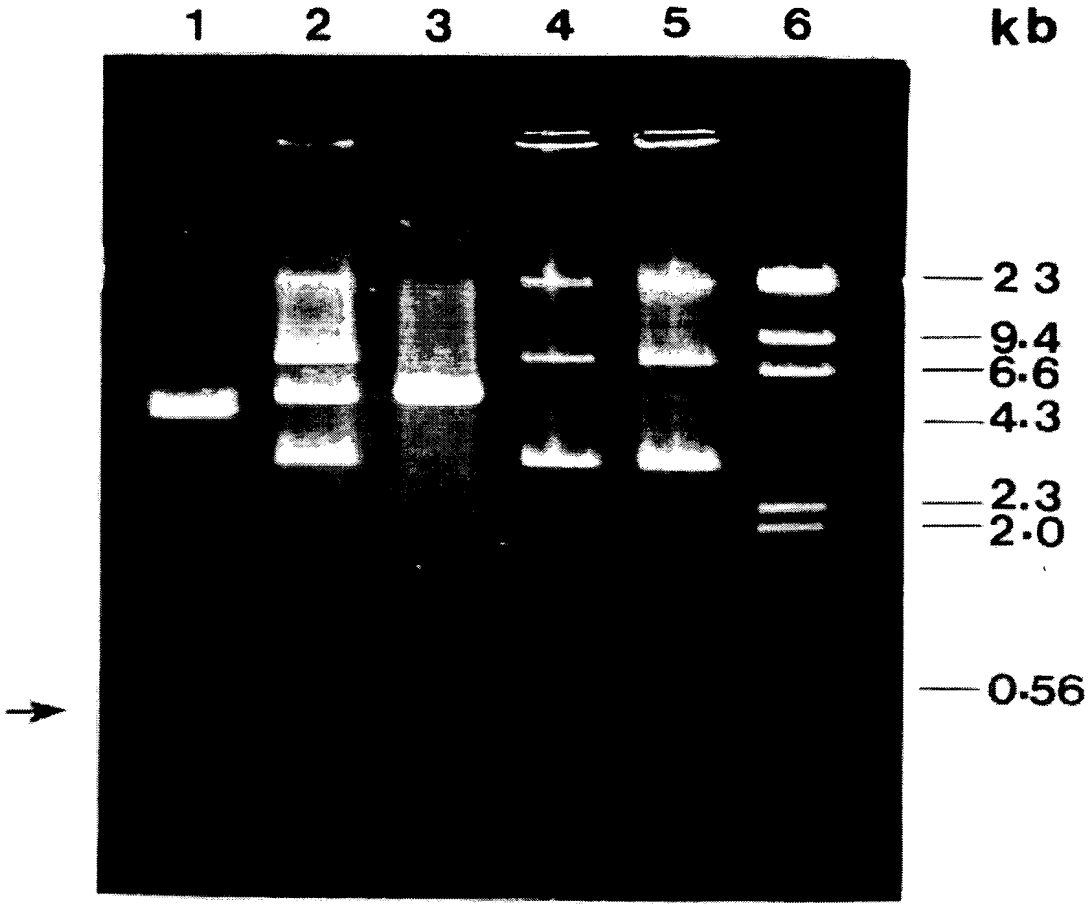


Fig. 2.5

DNA sequence of Tm 13.17 cDNA clone

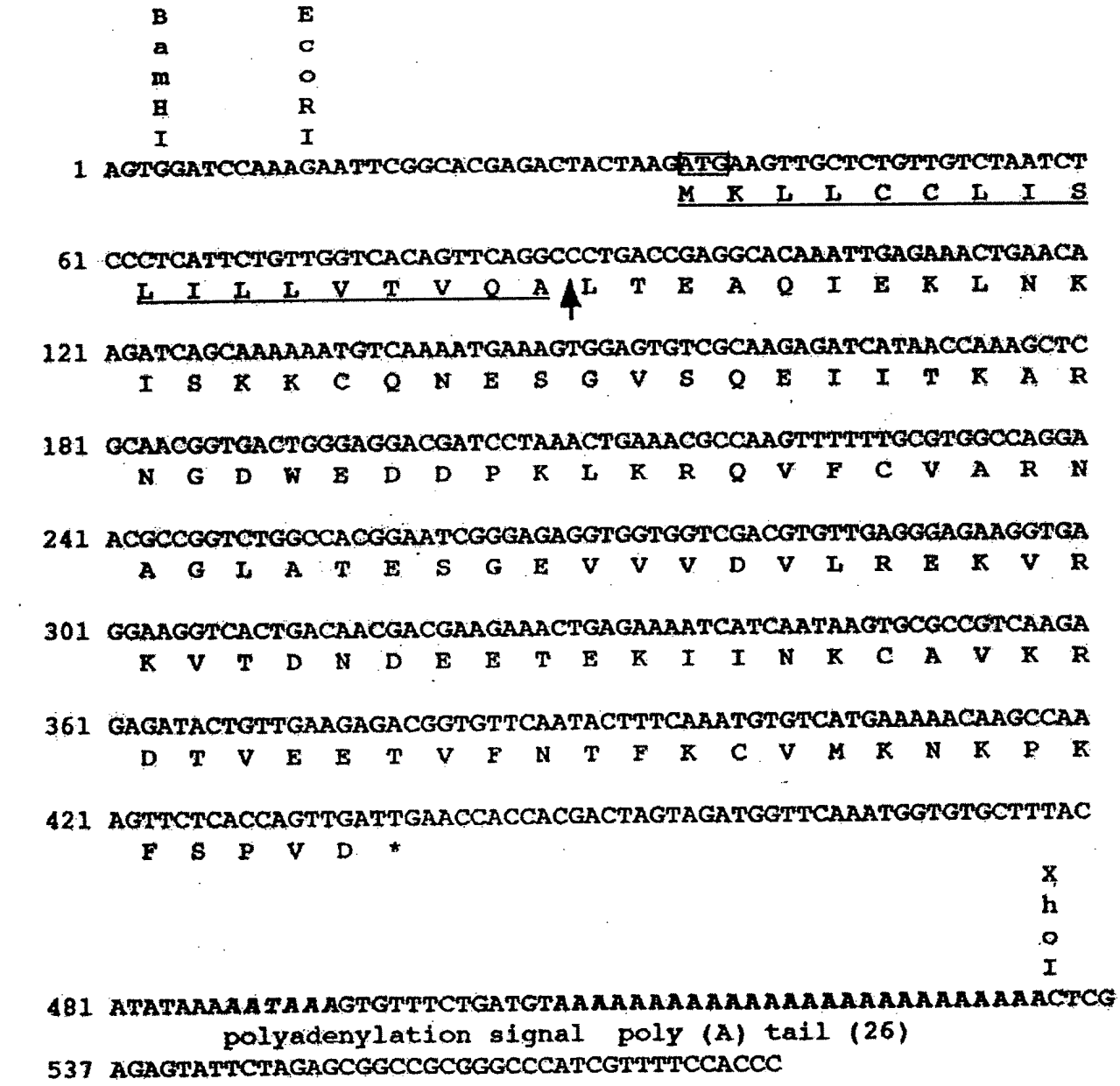


Fig. 2.6a

3037307 96.09.969

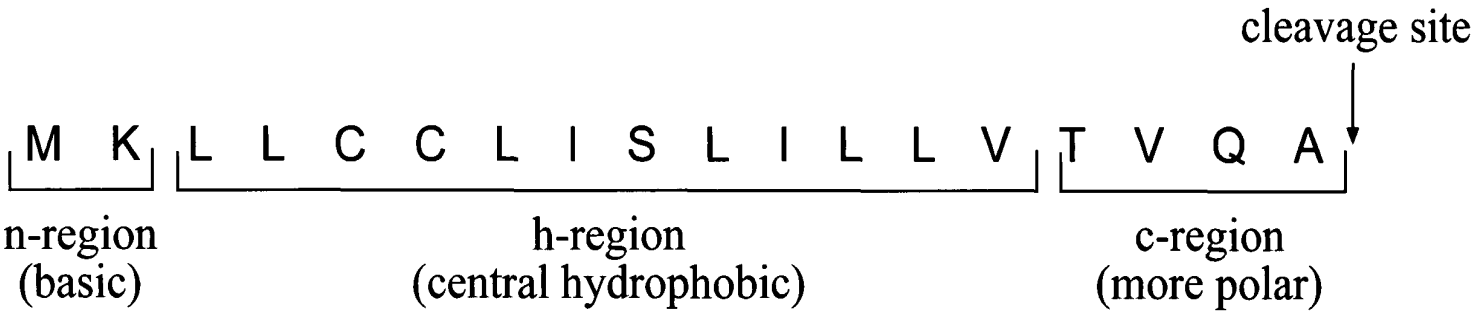


Fig. 2.6b

A. Mature Tm 13.17 amino acid r sidure

```
  1  LTEAQIEKLN KISKKCQNES GVSQEIITKA RNGDWEDDPK LKRQVFCVAR
 51  NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN
101  TFKCVMKNKP KFSPVD
```

B. Summary of the composition analysis for the mature Tm 13.17 sequence:

Residue	Number	Mole Percent
A = Ala	6	5.172
B = Asx	0	0.000
C = Cys	4	3.448
D = Asp	8	6.897
E = **Glu**	**13**	**11.207**
F = Phe	4	3.448
G = Gly	4	3.448
H = His	0	0.000
I = Ile	6	5.172
K =**Lys**	**16**	**13.793**
L = Leu	5	4.310
M = Met	1	0.862
N = Asn	8	6.897
P = Pro	3	2.586
Q = Gln	4	3.448
R = Arg	6	5.172
S = Ser	5	4.310
T = Thr	8	6.897
V =**Val**	**14**	**12.069**
W = Trp	1	0.862
Y = Tyr	0	0.000
Z = Glx	0	0.000

Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

Fig. 2.6c

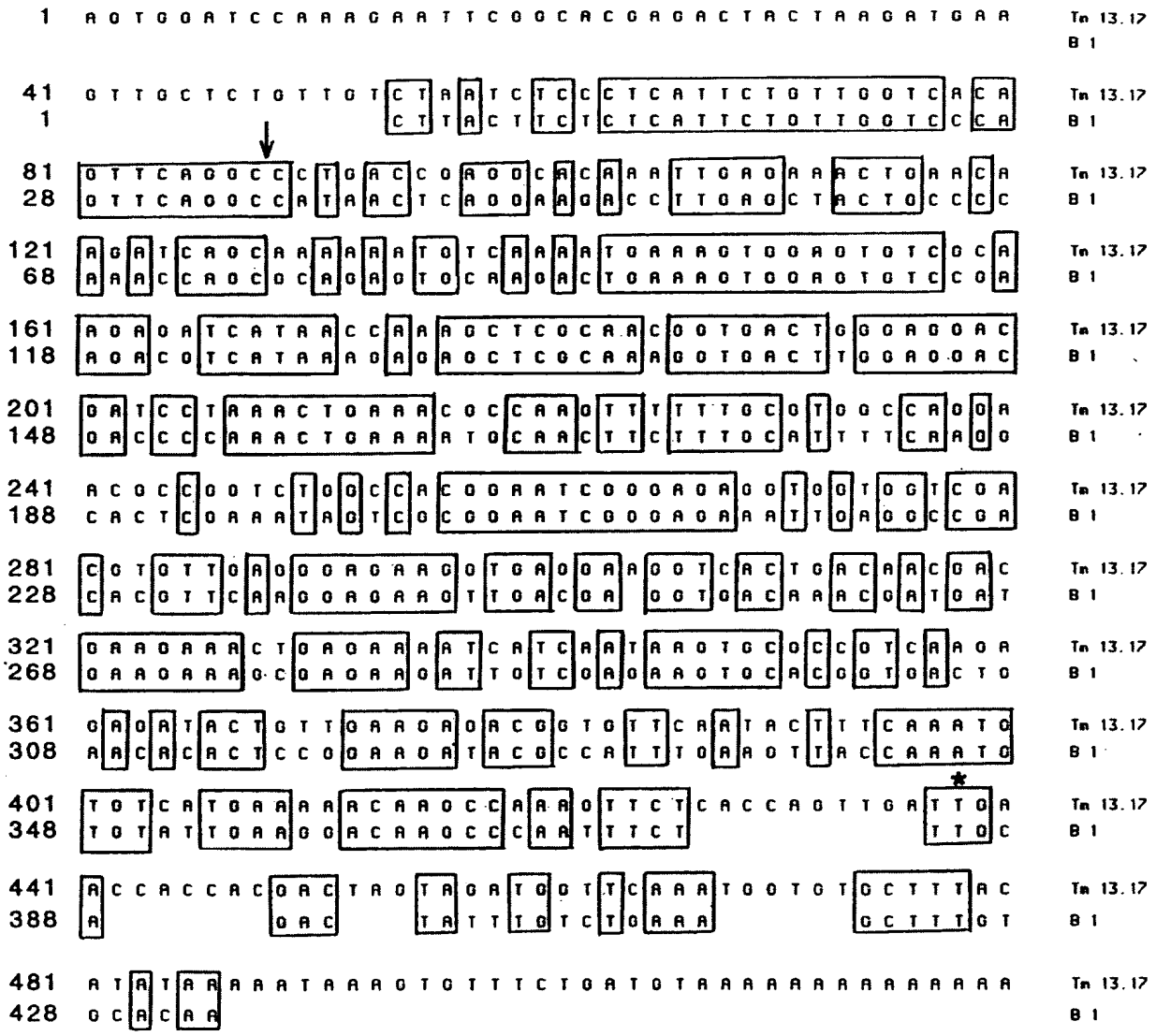

1 AGTGGATCCAAAGAATTCGGCACGAGACTACTAAGATGAA Tm 13.17 B 1
41 GTTGCTCTGTTGTCTAATCTCCTCATTCTGTTGGTCACA Tm 13.17 B 1
1 CTTACTTCTCATTCTGTTGGTCCCA
81 GTTCAGGCCCTGACCGAGDCACAAATTGAGAACTGAACA Tm 13.17 B 1
28 GTTCAGGCCCATAACTCAGDAAGACCTTGAGCTACTGCCCC
121 AGATCAGCAAAAAATGTCAAAATGAAAGTGGAGTGTCGCA Tm 13.17 B 1
68 AAACCAGCGCAGAGTGCAAGACTGAAAGTGGAGTGTCCGA
161 AGAGATCATAACCAAAGCTCGCAACGGTGACTGGGAGGAC Tm 13.17 B 1
118 AGACGTCATAAAGAGCTCGCAAAGGTGACTTGGAGGAC
201 GATCCTAAACTGAAACGCCAAGTTTTTGCGTGGCCAGGA Tm 13.17 B 1
148 GACCCAAACTGAAAATGCAACTTCTTTGCATTTCAAGG
241 ACGCCGGTCTGGCCAGCGAATCGGGAGAGGTGGTGGTCGA Tm 13.17 B 1
188 CACTCGAAATAGTCGCGGAATCGGGAGAAATTGAGGCCGA
281 CGTGTTGAGGGAGAAGGTGAAGAAGGTCACTGACAACGAC Tm 13.17 B 1
228 CACGTTCAAGGAGAAGTTGAACGAGGTGACAACGATGAT
321 GAAGAAACTGAAGAAAATCATCAAATAAGTGCGCCGTCAAGA Tm 13.17 B 1
268 GAAGAAAGCGAAGAAGATTGTCGAGAAGTGCACGGTGACTG
361 GAGATACTGTTGAAAGAGACGGGTGTTCAATACTTTCAAATG Tm 13.17 B 1
308 AACACACTCCGGAAAGATACGCCAATTTGAAAGTTACCAAATG
401 TGTCATGAAAAACAAGCCAAAGTTCTCACCAGTTGATTGA Tm 13.17 B 1
348 TGTATGAAGGACAAGCCCAATTCT TTGC
441 ACCACCACGACTAGTAGATGGTTCAAATGGGTGTTTTAC Tm 13.17 B 1
388 A GAC TATTTGTCTGAAA GCTTTGT
481 ATATAAAATAAAGTGTTTCTGATGTAAAAAAAAAAAAAAA Tm 13.17 B 1
428 GCACAA

Fig. 2.7

```
Tm 13.17      3  EAQIEKLNKISKKCQNESGVSQEIITKARNGDWEDDPKLKRQVFCVARNA 52
                 |.. |||.. |. |..|||||:| :.|.|| :  |||||| :.||: :.|
AFP-3         1  ETPREKLKQHSDACKAESGVSEESLNKVRNREEVDDPKLKEHAFCILKRA 50

Tm 13.17     53  GLATESGEVVVDVLREKVRKVTDNDEETEKIINKCAVKRDTVEETVFNTF 102
                 |: ..|||. :| ::.|.:. .::.|..:.::.|||||:||.:..  : |
AFP-3        51  GFIDASGEFQLDHIKTKFKENSEHPEKVDDLVAKCAVKKDTPQHSSADFF 100

Tm 13.17    103  KCVMKNKP 110
                 ||| .|:.
AFP-3       101  KCVHDNRS 108
```

Percent identity: 39.8 (identical amino acids; Percent similarity: 58.3 (identical amino acids plus conservative amino acids).

Fig. 2.8

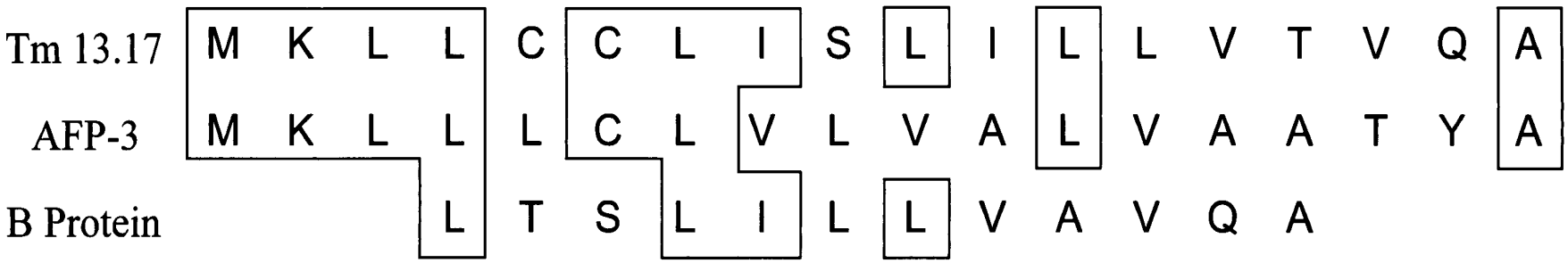

Tm 13.17
M K L L C C L I S L I L L V T V Q A
AFP-3
M K L L L C L V L V A L V A A T Y A
B Protein
L T S L I L L V A V Q A

Fig. 2.9

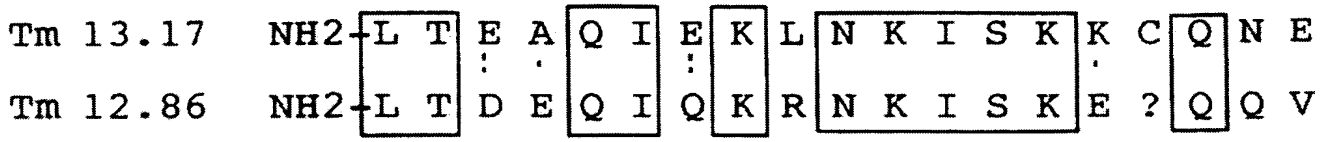


Fig 2.10

09/07/95 02:14:02

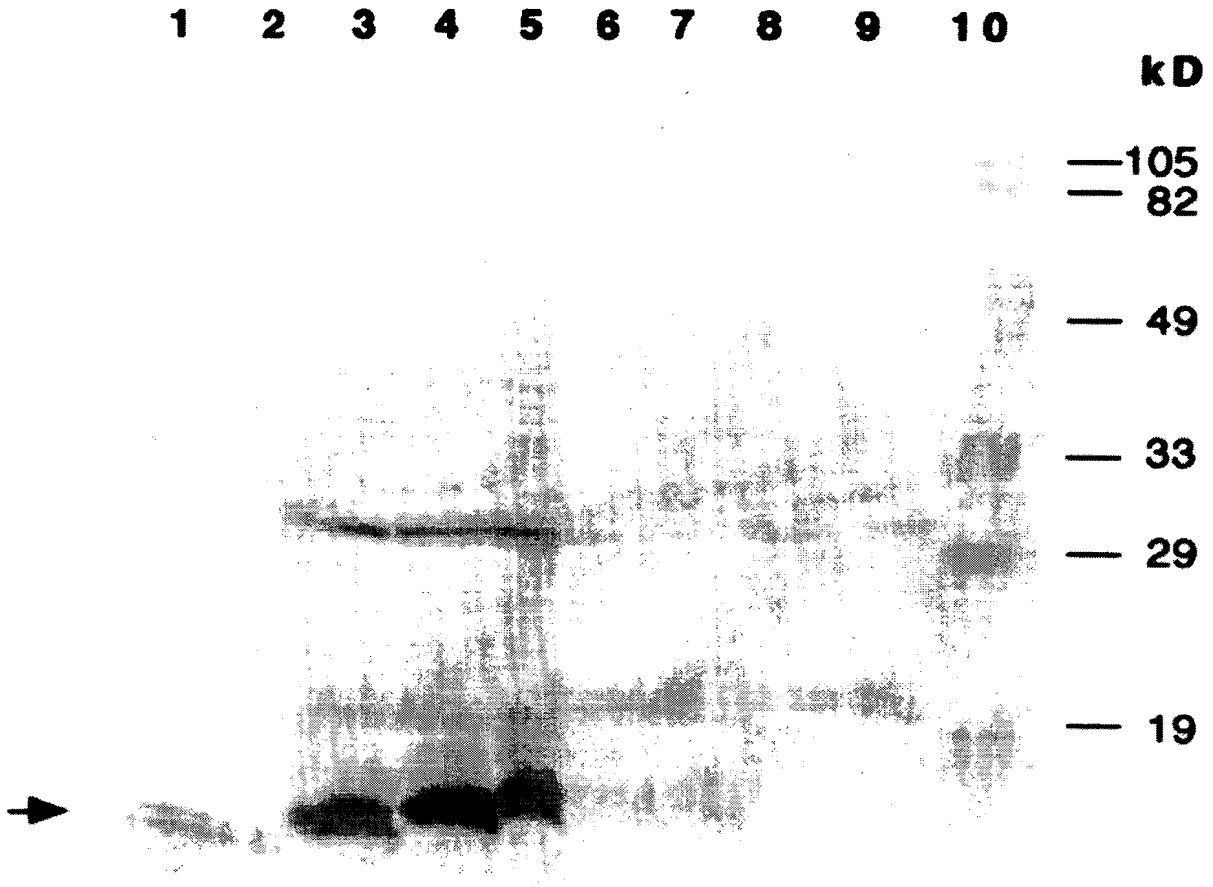


Fig 2.11

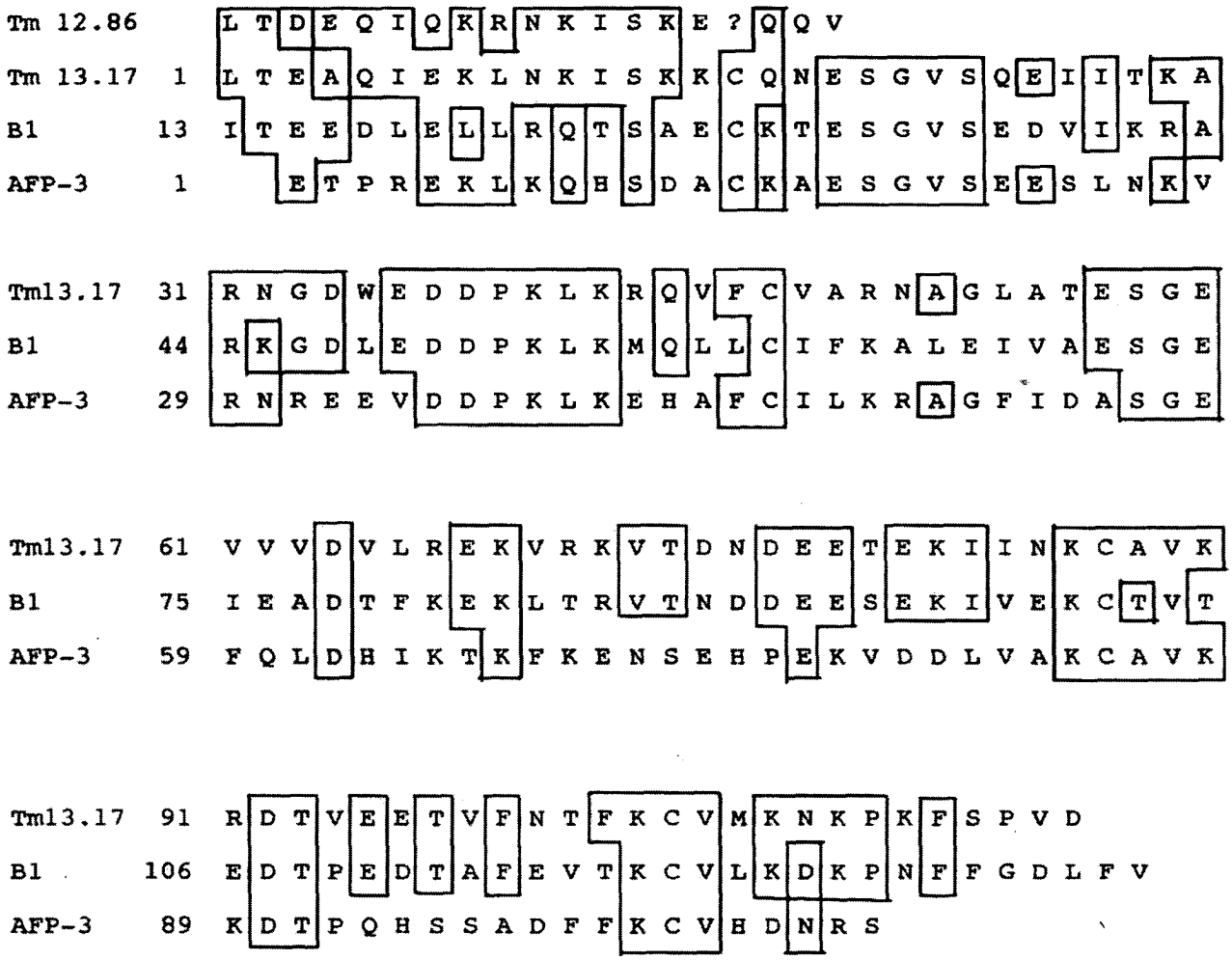

Tm 12.86 LTDEQIQKRNKISKE?QQV
Tm 13.17 1 LTEAQIEKLNKISKKCQNESGVSQEIITKA
B1 13 ITEEDLELLRQTSAECKTESGVSEDVIKRA
AFP-3 1 ETPREKLKQHSDACKAESGVSEESLNKV
Tm13.17 31 RNGDWEDDPKLKRQVFCVARNAGLATESGE
B1 44 RKGDLEDDPKLKMQLLCIFKALEIVAESGE
AFP-3 29 RNREEVDDPKLKEHAFCILKRAGFIDASGE
Tm13.17 61 VVVDVLREKVRKVTDNDEETEKIINKCAVK
B1 75 IEADTFKEKLTRVTNDDEESEKIVEKCTVT
AFP-3 59 FQLDHIKTKFKENSEHPEKVDDLVAKCAVK
Tm13.17 91 RDTVEETVFNTFKCVMKNKPKFSPVD
B1 106 EDTPEDTAFEVTKCVLKDKPNFFGDLFV
AFP-3 89 KDTPQHSSADFFKCVHDNRS

Fig. 2.12

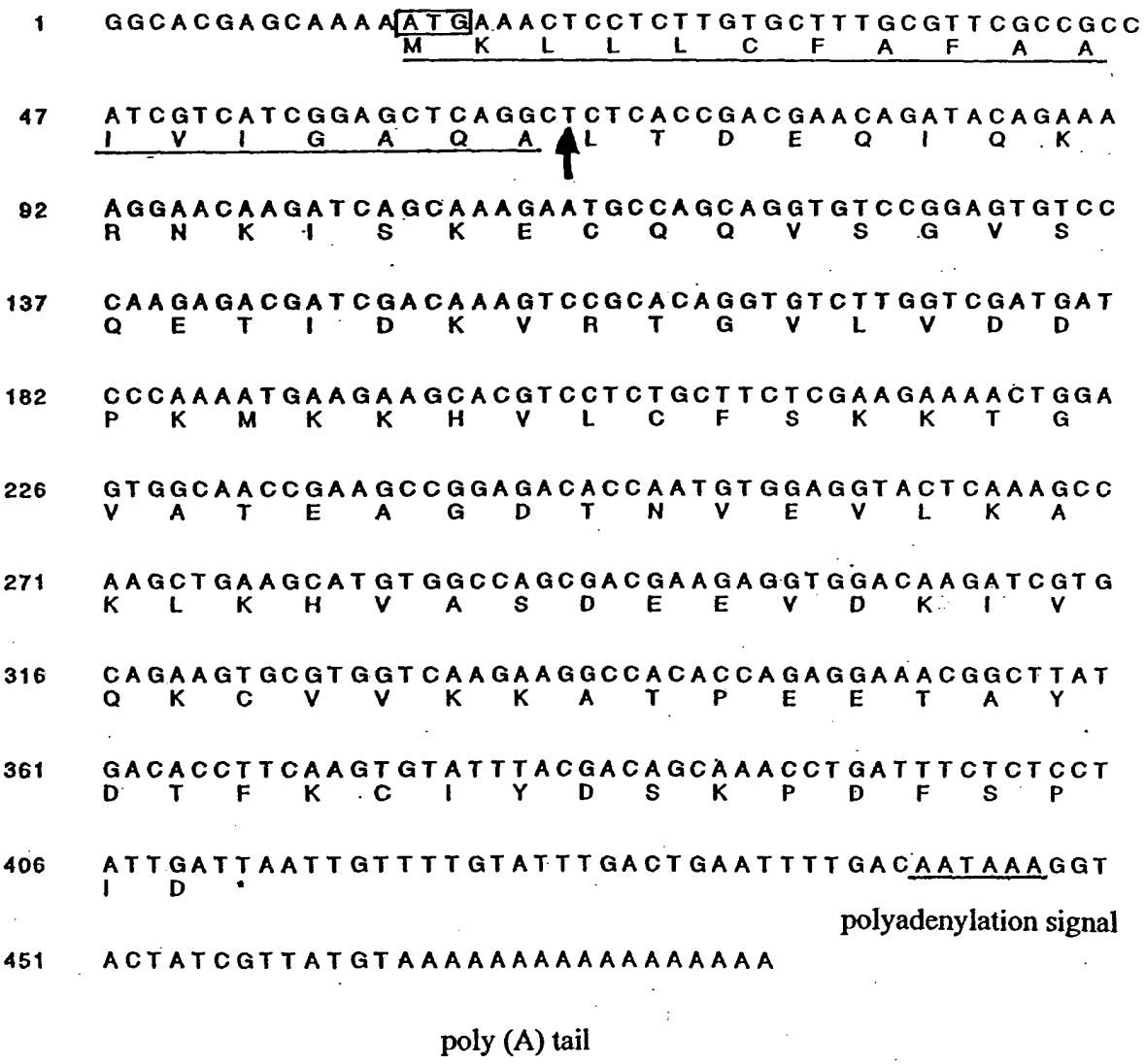

1 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC
M K L L L C F A F A A
47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
I V I G A Q A L T D E Q I Q K
92 AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
R N K I S K E C Q Q V S G V S
137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGATGAT
Q E T I D K V R T G V L V D D
182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
P K M K K H V L C F S K K T G
226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
V A T E A G D T N V E V L K A
271 AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG
K L K H V A S D E E V D K I V
316 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
Q K C V V K K A T P E E T A Y
361 GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCTCTCCT
D T F K C I Y D S K P D F S P
406 ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
I D *
polyadenylation signal
451 ACTATCGTTATGTAAAAAAAAAAAAAAAA
poly (A) tail

Fig. 3.0

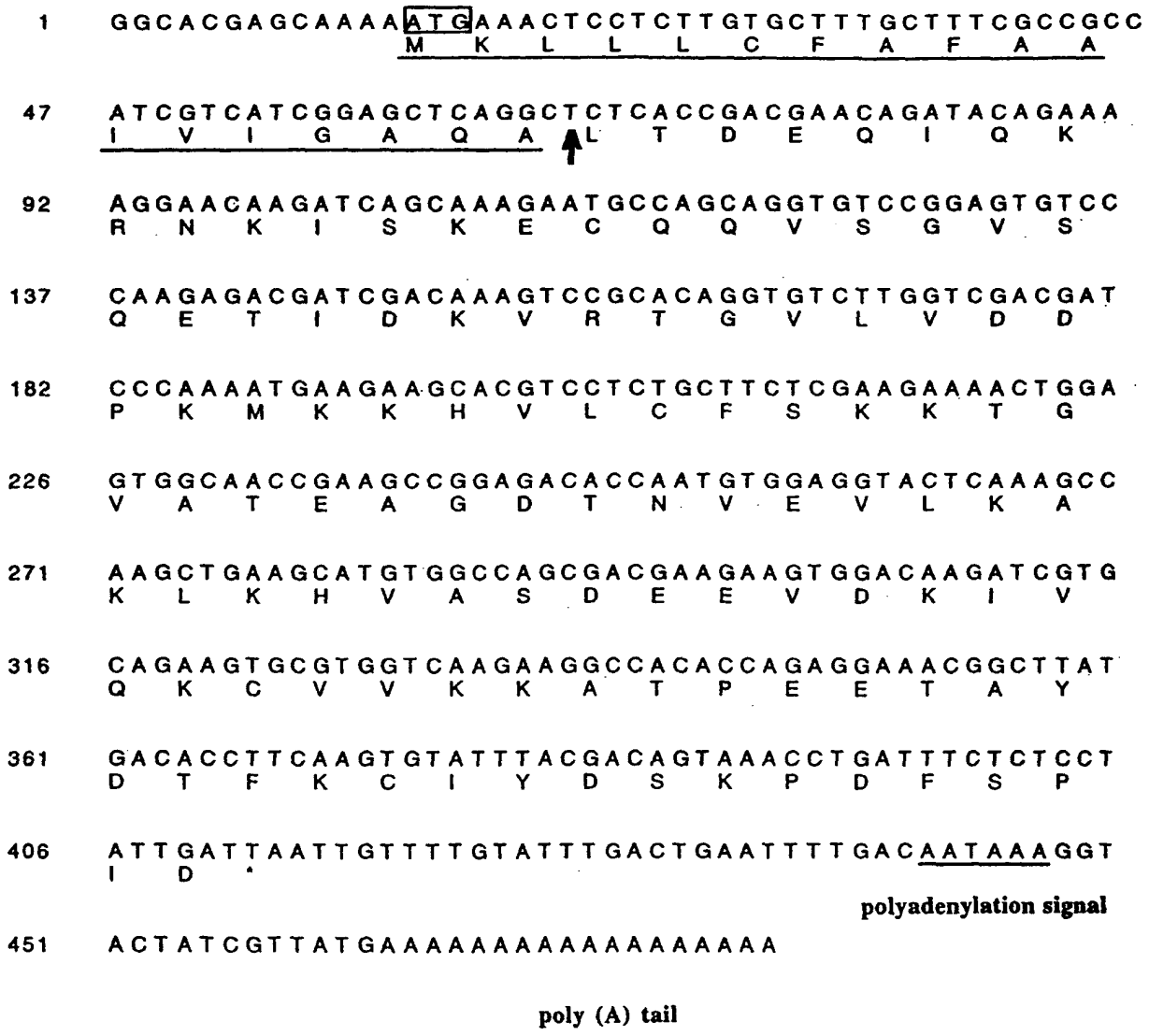

1 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCC
M K L L L C F A F A A
47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
I V I G A Q A L T D E Q I Q K
92 AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCCGGAGTGTCC
R N K I S K E C Q Q V S G V S
137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
Q E T I D K V R T G V L V D D
182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
P K M K K H V L C F S K K T G
226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
V A T E A G D T N V E V L K A
271 AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
K L K H V A S D E E V D K I V
316 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
Q K C V V K K A T P E E T A Y
361 GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
D T F K C I Y D S K P D F S P
406 ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
I D *
polyadenylation signal
451 ACTATCGTTATGAAAAAAAAAAAAAAAAA
poly (A) tail

Fig 3.1

Start
2-2 GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCG
2-3 GGCACGAGCAAAAAATGAAACTCCTCTTGTGCTTTGCT
2-2 TTCGCCGCCATCGTCATCGGAGCTCAGGCTCTCACCG
2-3 TTCGCCGCCATCGTCATCGGAGCTCAGGCTCTCACCG
2-2 ACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGA
2-3 ACGAACAGATACAGAAAAGGAACAAGATCAGCAAAGA
2-2 ATGCCAGCAGGTGTCCGGAGTGTCCCAAGAGACGATC
2-3 ATGCCAGCAGGTGTCCGGAGTGTCCCAAGAGACGATC
2-2 GACAAAGTCCGCACAGGTGTCTTGGTCGATGATCCCA
2-3 GACAAAGTCCGCACAGGTGTCTTGGTCGACGATCCCA
2-2 AAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAAC
2-3 AAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAAC
2-2 TGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAG
2-3 TGGAGTGGCAACCGAAGCCGGAGACACCAATGTGGAG
2-2 GTACTCAAAGCCAAGCTGAAGCATGTGGCCAGCGACG
2-3 GTACTCAAAGCCAAGCTGAAGCATGTGGCCAGCGACG
2-2 AAGAGGTGGACAAGATCGTGCAGAAGTGCGTGGTCAA
2-3 AAGAAGTGGACAAGATCGTGCAGAAGTGCGTGGTCAA
2-2 GAAGGCCACACCAGAGGAAACGGCTTATGACACCTTC
2-3 GAAGGCCACACCAGAGGAAACGGCTTATGACACCTTC
2-2 AAGTGTATTTACGACAGCAAACCTGATTTCTCTCCTA
2-3 AAGTGTATTTACGACAGTAAACCTGATTTCTCTCCTA
2-2 TTGATTAATTGTTTTGTATTTGACTGAATTTTGACAA
2-3 TTGATTAATTGTTTTGTATTTGACTGAATTTTGACAA
2-2 TAAAGGTAATATCGTTATGTAAAAA
2-3 TAAAGGTACTATCGTTATGAAAAAA

Fig 3.2

Predicted Amino Acid

Composition of 2-2 and 2-3

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.19	41.74
Acidic (DE)	20	18.90	17.39
Basic (KR)	20	20.40	17.39
Polar (NCQSTY)	30	25.35	26.09
Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

Fig. 3.3

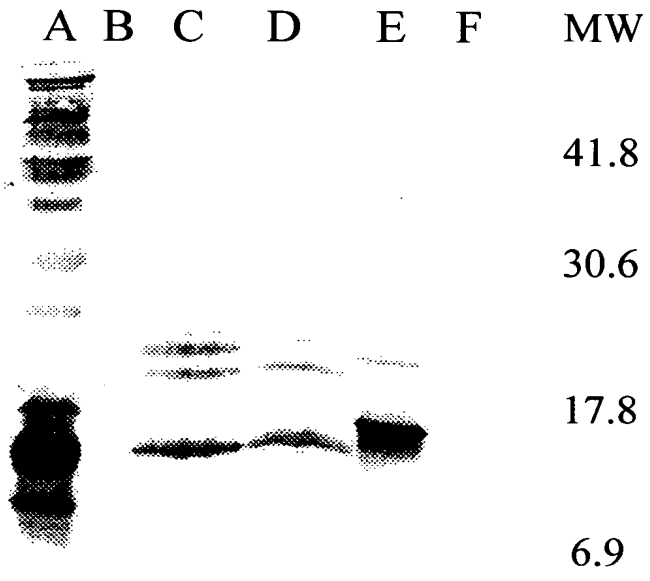


Fig. 3.4

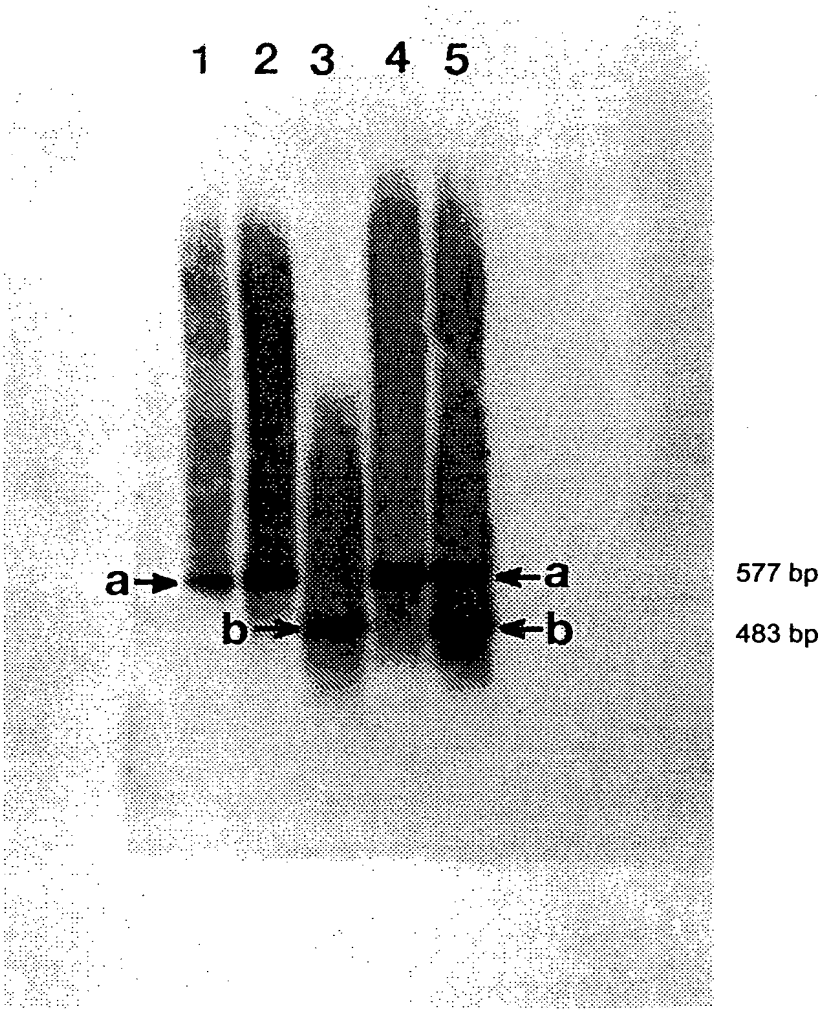


Fig. 4.0

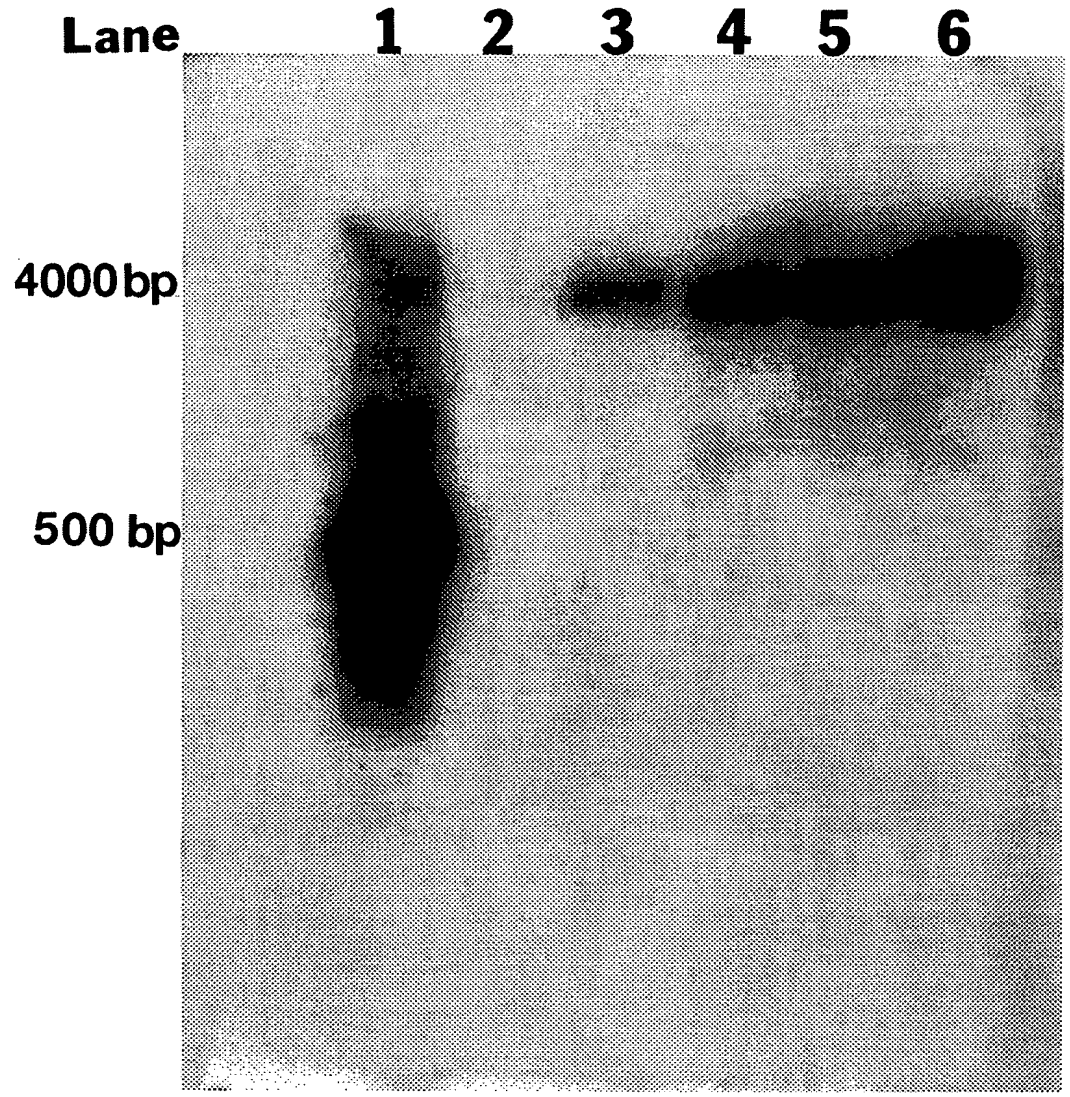


Fig. 4.1

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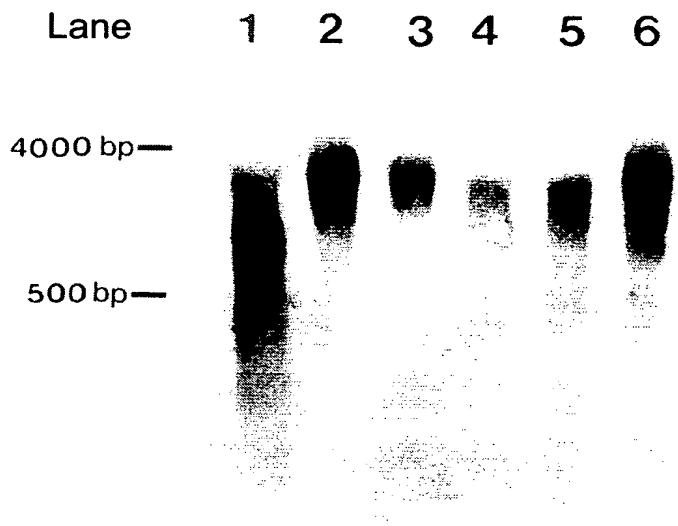


Fig. 4.2

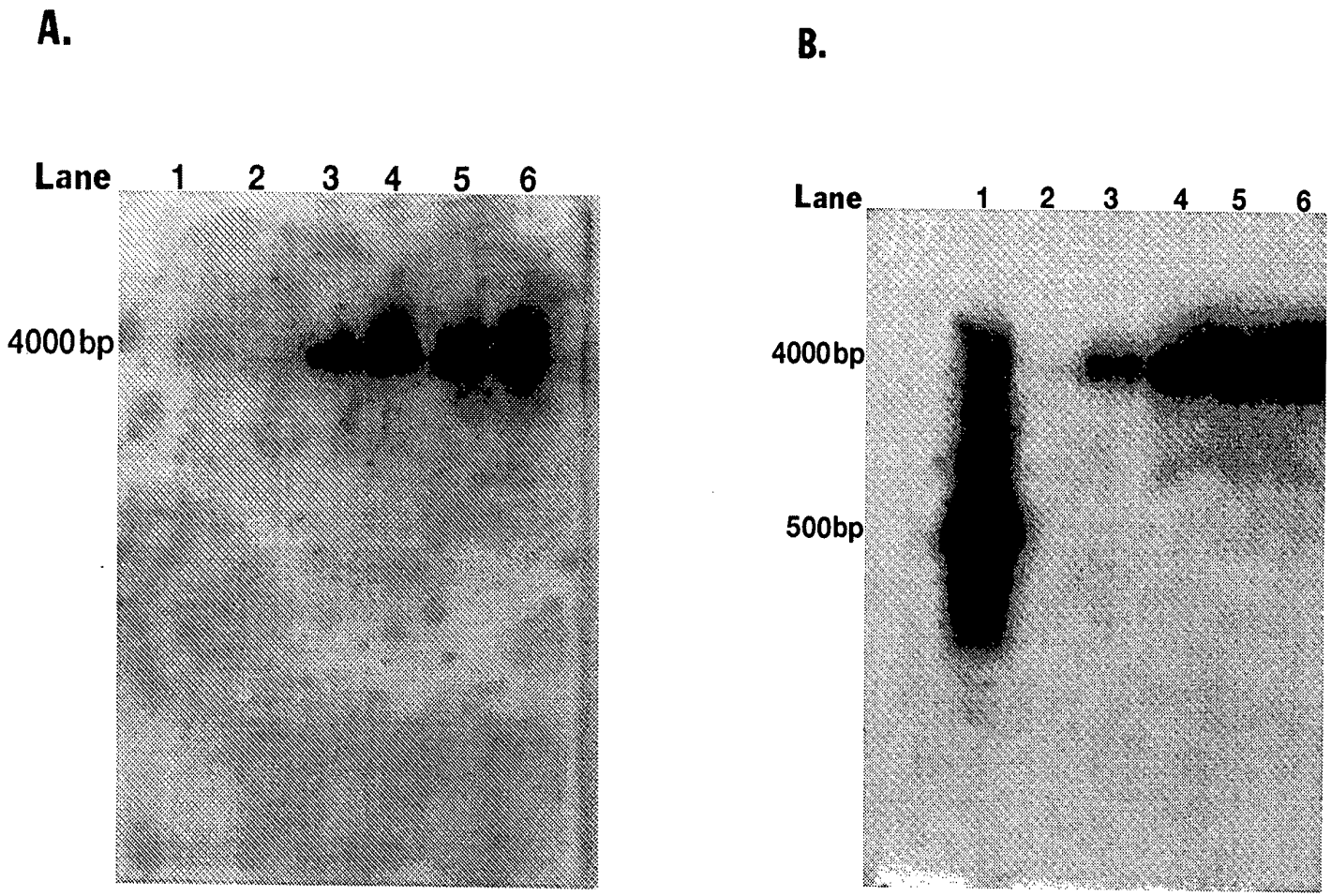


Fig. 4.3

Sequence: 6 of 6 on 2/4/2005

Fig. 4.4

Document "Confidential"

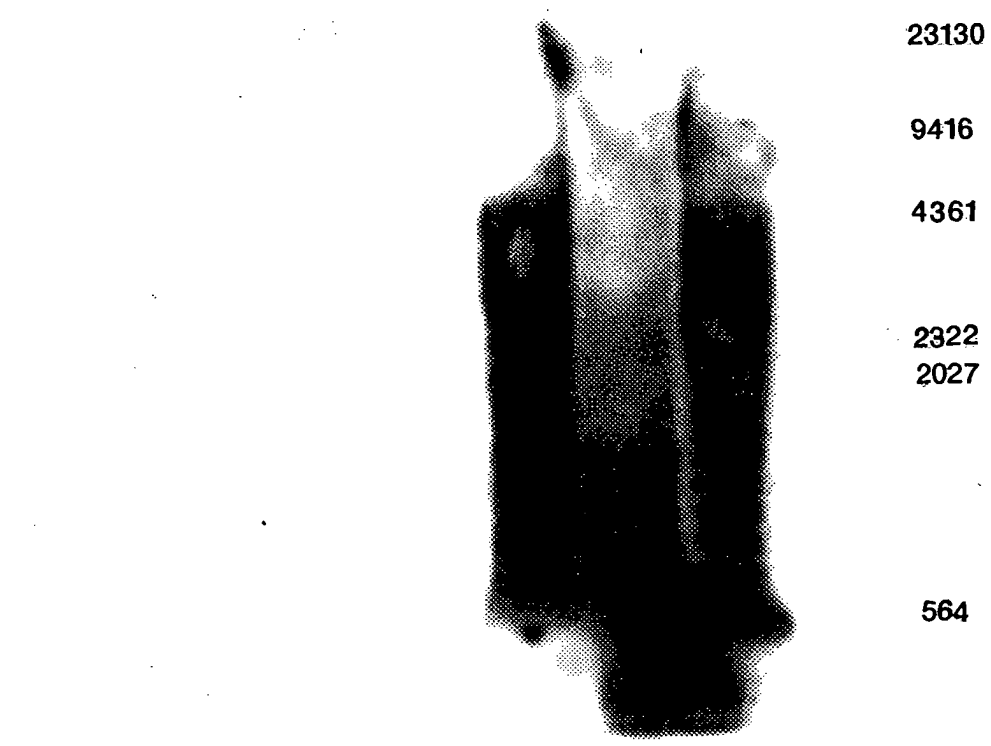


Fig. 4.5.

Tm 13.17 cDNA

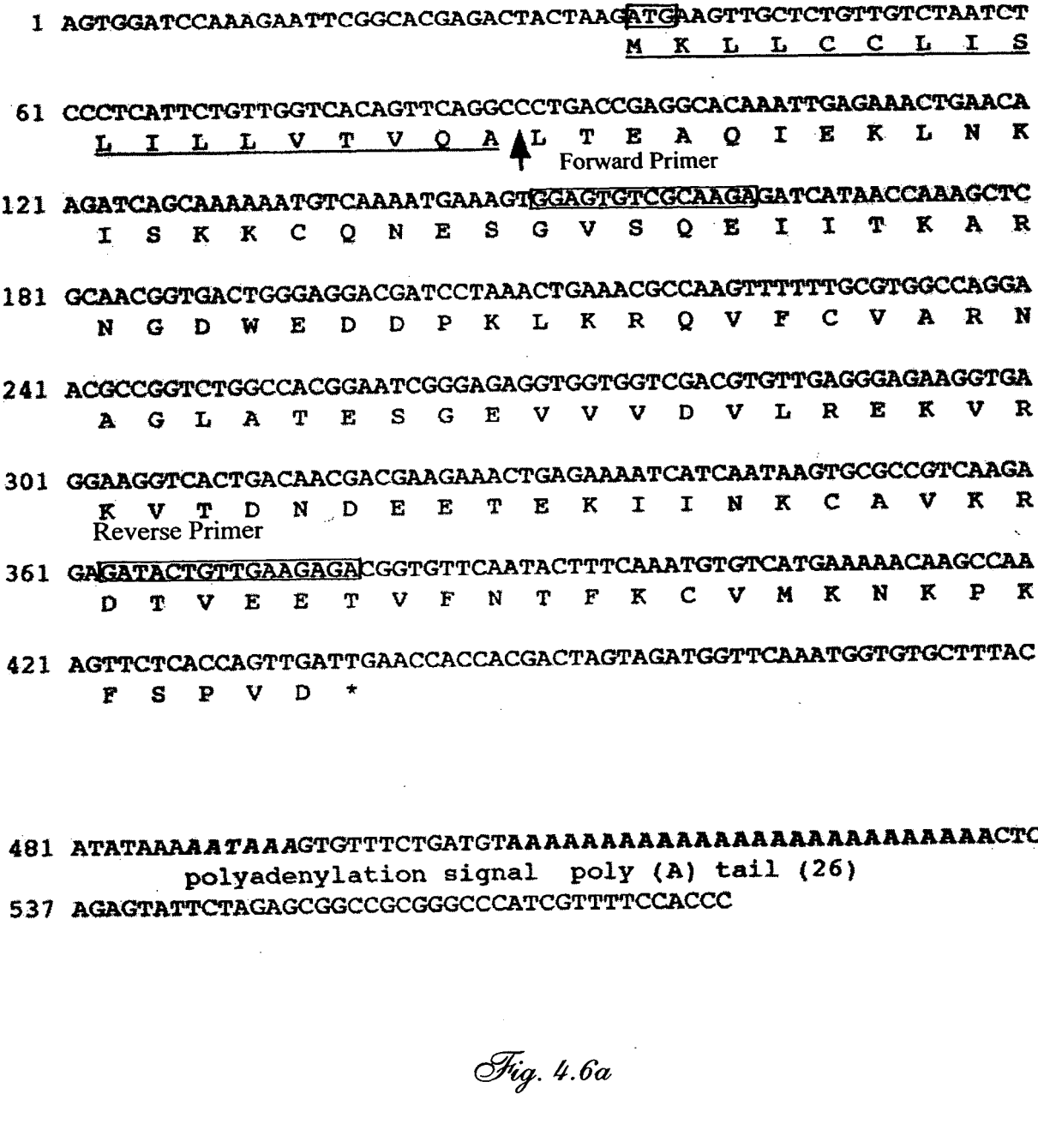


Fig. 4.6a

Forward Primer

```
2-2       L T D E Q I Q K R N K I S K E C Q Q V S G V S Q E T I D K V R T G V L V
Tm 13.17  L T E A Q I E K L N K I S K K C Q N E S G V S Q E I I T K A R N G D W E
B2        L T E E D L Q L L R Q T S A E C K T E S G A S E A V I K K A R K G D L E
AFP-3       E T P R E K L K Q H S D A C K A E S G V S E E S L N K V R N R E E V

2-2       D D P K M K K H V L C F S K K T G V A T E A G D T N V E V L K A K L K H
Tm 13.17  D D P K L K R Q V F C V A R N A G L A T E S G E V V V D V L R E K V R K
B2        D D P K L K M Q L L C I F K A L E I V A E S G E I E A D T F K E K L T R
AFP-3     D D P K L K E H A F C I L K R A G F I D A S G E F Q L D H I K T K F K E
```

Reverse Primer

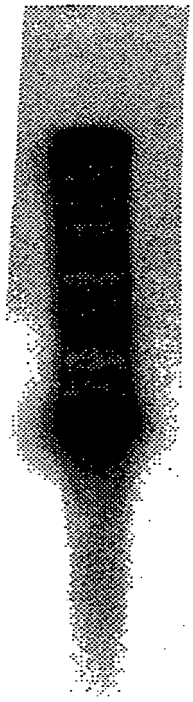
```
2-2       V A S   D E E V D K I V Q K C V V K K A T P E E T A Y D T F K C I Y D S
Tm 13.17  V T D N D E E T E K I I N K C A V K R D T V E E T V F N T F K C V M K N
B2        V T N D D E E S E K I V E K C T V T E D T P E D T A F E V T K C V L K D
AFP-3     N S E H P E K V D D L V A K C A V K K D T P Q H S S A D F F K C V H D N

2-2       K P D F S P I D
Tm 13.17  K P K F S P V D
B2        K P N F F G D L F V
AFP-3     R S
```

Fig. 4.6b

	percent % composition				
Primer	A	C	G	T	MeltingTemperature(°C)
Forward	28.6	14.3	42.9	14.3	44.0
Reverse	25.0	31.3	6.3	37.5	44.0

Fig. 4.6c

Sheet 5 of 9 09/762,459



3600 bp

Fig. 4.7

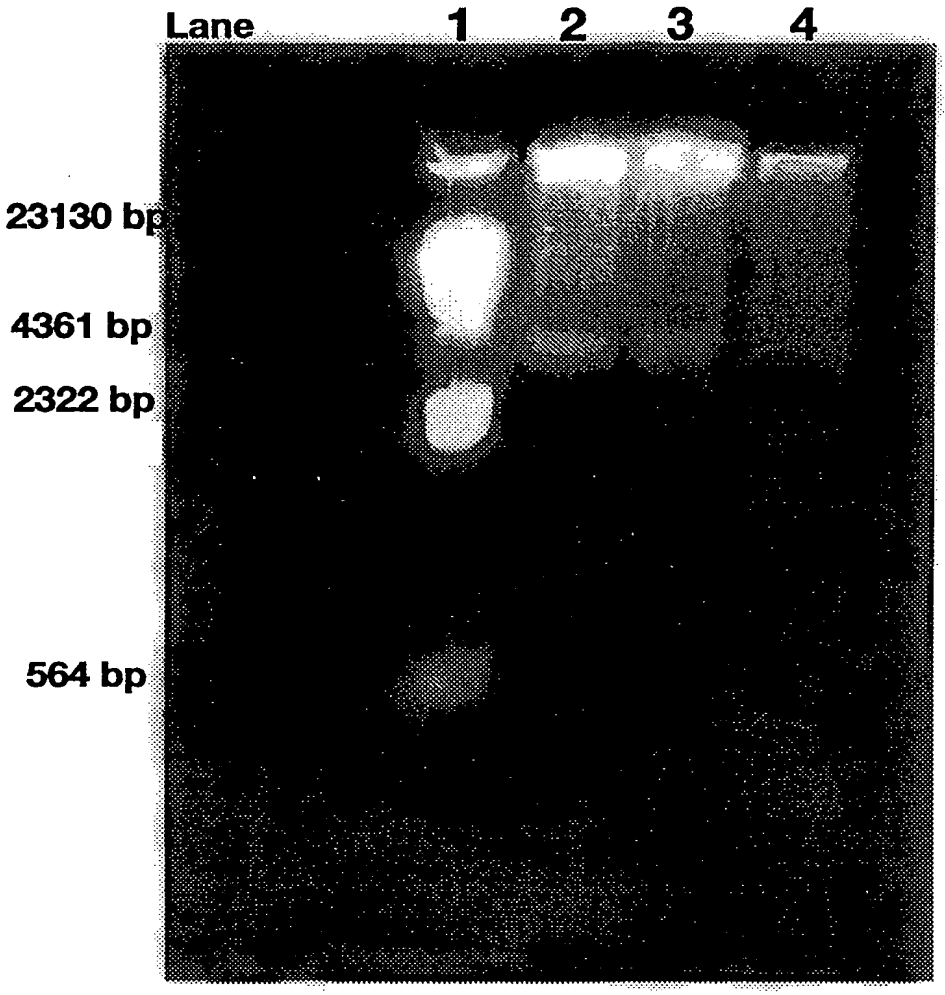


Fig. 4.8

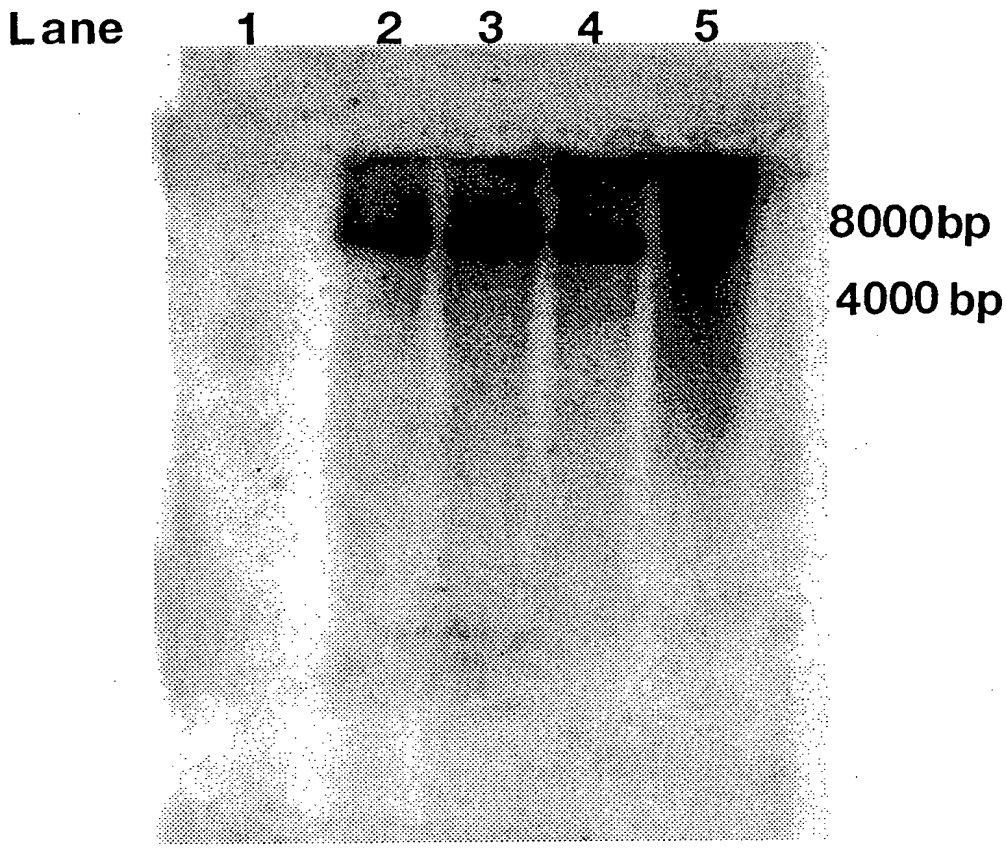


Fig. 4.9

Docket No. 5050-5

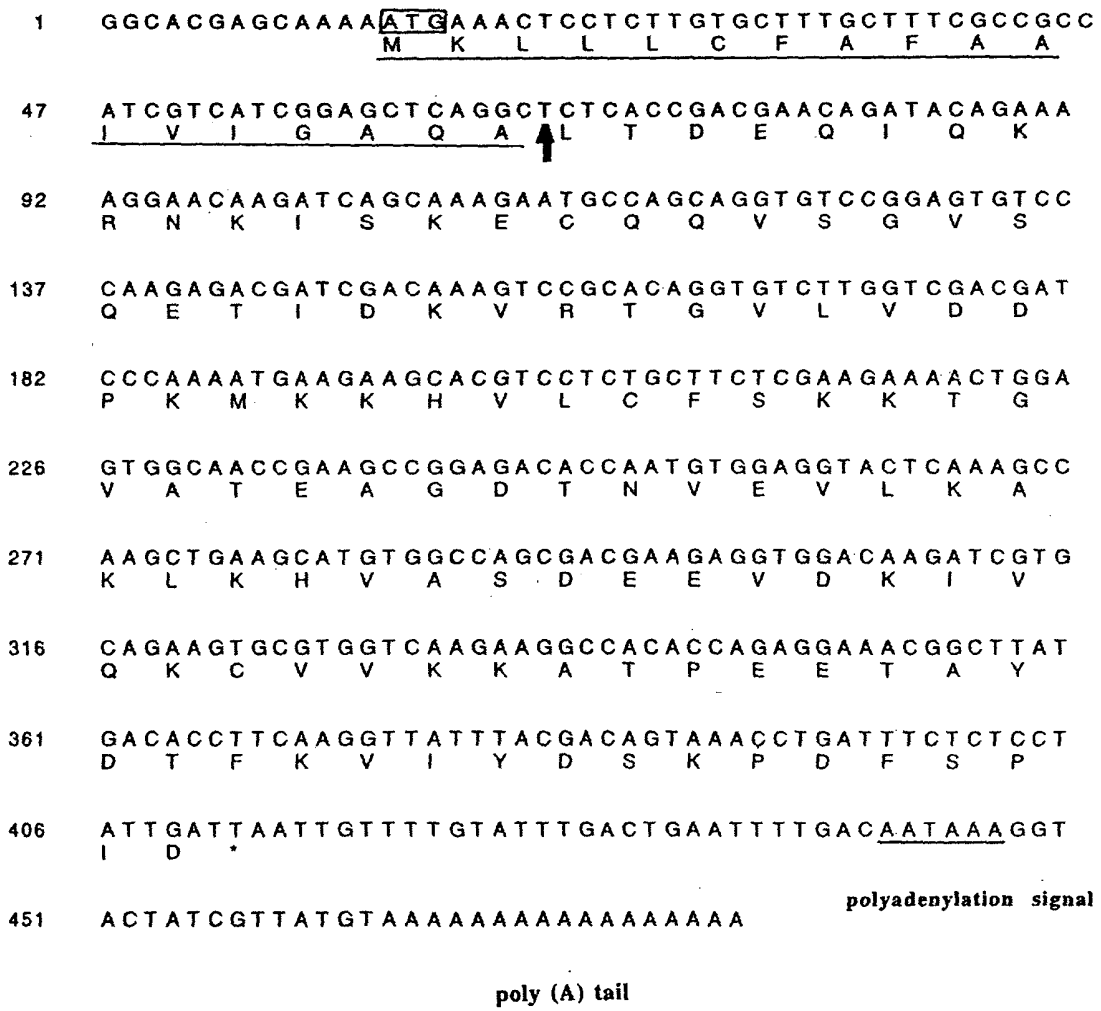


Fig. 4.10a

Predicted Amino Acid

Composition of 3-4

Molecular Weight	12839.70 m.w.
Length	115
1 microgram =	77.883 pMoles
Molar Extinction coefficient	2920±5%
1 A(280) =	4.40 mg/ml
Isoelectric Point	7.14
Charge at pH 7	0.16

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	47	46.41	40.87
Acidic (DE)	20	18.91	17.39
Basic (KR)	20	20.41	17.39
Polar (NCQSTY)	29	24.55	25.22
Hydrophobic (AILFWV)	35	28.04	30.43
A Ala	6	3.32	5.22
C Cys	3	2.41	2.61
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.99	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.09	7.83
V Val	15	11.58	13.04
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

Fig. 4.10b

05/03/2001 - 03:04:55 PM

```
1    GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCTTTCGCCGCC
                M  K  L  L  L  C  F  A  F  A  A

47   ATCGTCATCGGAGCTCAGGCTCTCACCGATGAACAGATACAGAAA
     I  V  I  G  A  Q  A  L  T  D  E  Q  I  Q  K

92   AGGAACAAGATCAGCAAAGAATGCCAGCAGGAGTCCGGAGTGTCC
     R  N  K  I  S  K  E  C  Q  Q  E  S  G  V  S

137  CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
     Q  E  T  I  D  K  V  R  T  G  V  L  V  D  D

182  CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAGAACTGGA
     P  K  M  K  K  H  V  L  C  F  S  K  R  T  G

226  GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
     V  A  T  E  A  G  D  T  N  V  E  V  L  K  A

271  AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
     K  L  K  H  V  A  S  D  E  E  V  D  K  I  V

316  CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
     Q  K  C  V  V  K  K  A  T  P  E  E  T  A  Y

361  GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
     D  T  F  K  V  I  Y  D  S  K  P  D  F  S  P

406  ATTGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
     I  D  *
                                          polyadenylation signal

451  ACTATCGTTATGAAAAAAAAAAAAAAAAAA

                poly (A) tail
```

Fig. 4.11a

Predicted Amino Acid

Composition of 3-9

Analysis	Whole Protein
Molecular Weight	12871.80 m.w.
Length	115
1 microgram =	77.689 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.23 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

Amino Acid(s)	Number count	% by weight	% by frequency
Charged (RKHYCDE)	48	47.31	41.74
Acidic (DE)	20	18.86	17.39
Basic (KR)	20	20.57	17.39
Polar (NCQSTY)	30	25.29	26.09
Hydrophobic (AILFWV)	34	27.20	29.57
A Ala	6	3.31	5.22
C Cys	4	3.21	3.48
D Asp	11	9.84	9.57
E Glu	9	9.03	7.83
F Phe	3	3.43	2.61
G Gly	4	1.77	3.48
H His	2	2.13	1.74
I Ile	6	5.28	5.22
K Lys	17	16.93	14.78
L Leu	5	4.40	4.35
M Met	1	1.02	0.87
N Asn	2	1.77	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.97	5.22
R Arg	3	3.64	2.61
S Ser	7	4.74	6.09
T Thr	9	7.07	7.83
V Val	14	10.78	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

Fig. 4.11b

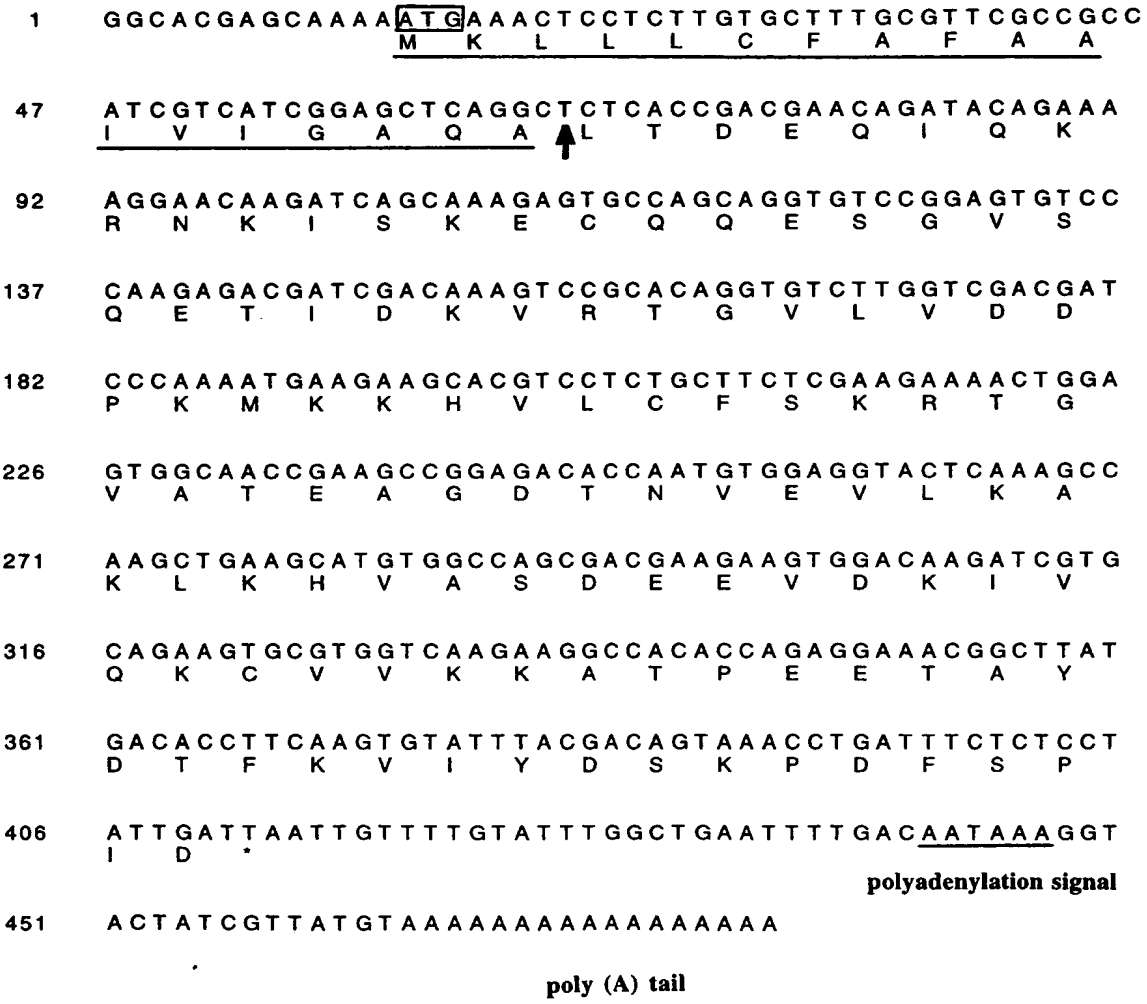

1 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCCGCC
M K L L L C F A F A A
47 ATCGTCATCGGAGCTCAGGCTCTCACCGACGAACAGATACAGAAA
I V I G A Q A L T D E Q I Q K
92 AGGAACAAGATCAGCAAAGAGTGCCAGCAGGTGTCCGGAGTGTCC
R N K I S K E C Q Q E S G V S
137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGACGAT
Q E T I D K V R T G V L V D D
182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
P K M K K H V L C F S K R T G
226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
V A T E A G D T N V E V L K A
271 AAGCTGAAGCATGTGGCCAGCGACGAAGAAGTGGACAAGATCGTG
K L K H V A S D E E V D K I V
316 CAGAAGTGCGTGGTCAAGAAGGCCACACCAGAGGAAACGGCTTAT
Q K C V V K K A T P E E T A Y
361 GACACCTTCAAGTGTATTTACGACAGTAAACCTGATTTCTCTCCT
D T F K V I Y D S K P D F S P
406 ATTGATTAATTGTTTTGTATTTGGCTGAATTTTGACAATAAAGGT
I D *
polyadenylation signal
451 ACTATCGTTATGTAAAAAAAAAAAAAAAAA
poly (A) tail

Fig. 4.12a

Predicted Amino Acid

Composition of 7–5

Analysis	Whole Protein
Molecular Weight	12843.80 m.w.
Length	115
1 microgram =	77.859 pMoles
Molar Extinction coefficient	3040±5%
1 A(280) =	4.22 mg/ml
Isoelectric Point	7.11
Charge at pH 7	0.13

Whole Protein Composition Analysis

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Hydrophobic (AILFWV)	34	27.26	29.57
A Ala	6	3.32	5.22
C Cys	4	3.21	3.48
D Asp	11	9.86	9.57
E Glu	9	9.05	7.83
F Phe	3	3.44	2.61
G Gly	4	1.78	3.48
H His	2	2.14	1.74
I Ile	6	5.29	5.22
K Lys	18	17.97	15.65
L Leu	5	4.41	4.35
M Met	1	1.02	0.87
N Asn	2	1.78	1.74
P Pro	4	3.02	3.48
Q Gln	6	5.98	5.22
R Arg	2	2.43	1.74
S Ser	7	4.75	6.09
T Thr	9	7.08	7.83
V Val	14	10.80	12.17
W Trp	0	0.00	0.00
Y Tyr	2	2.54	1.74
B Asx	0	0.00	0.00
Z Glx	0	0.00	0.00
X Xxx	0	0.00	0.00
. Ter	0	0.00	0.00

Fig. 4.12b

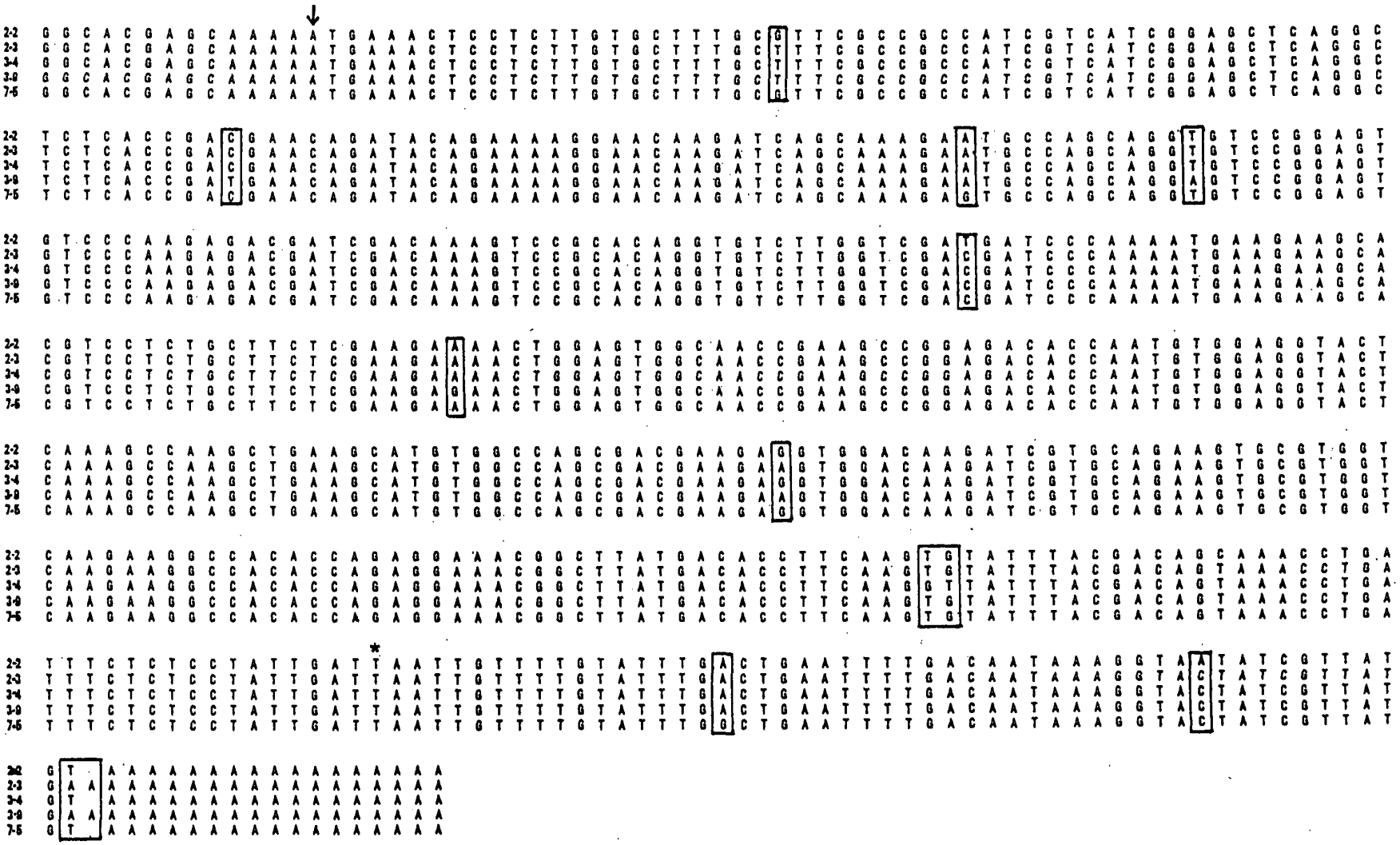

Fig 4.13

3037307 96/03763

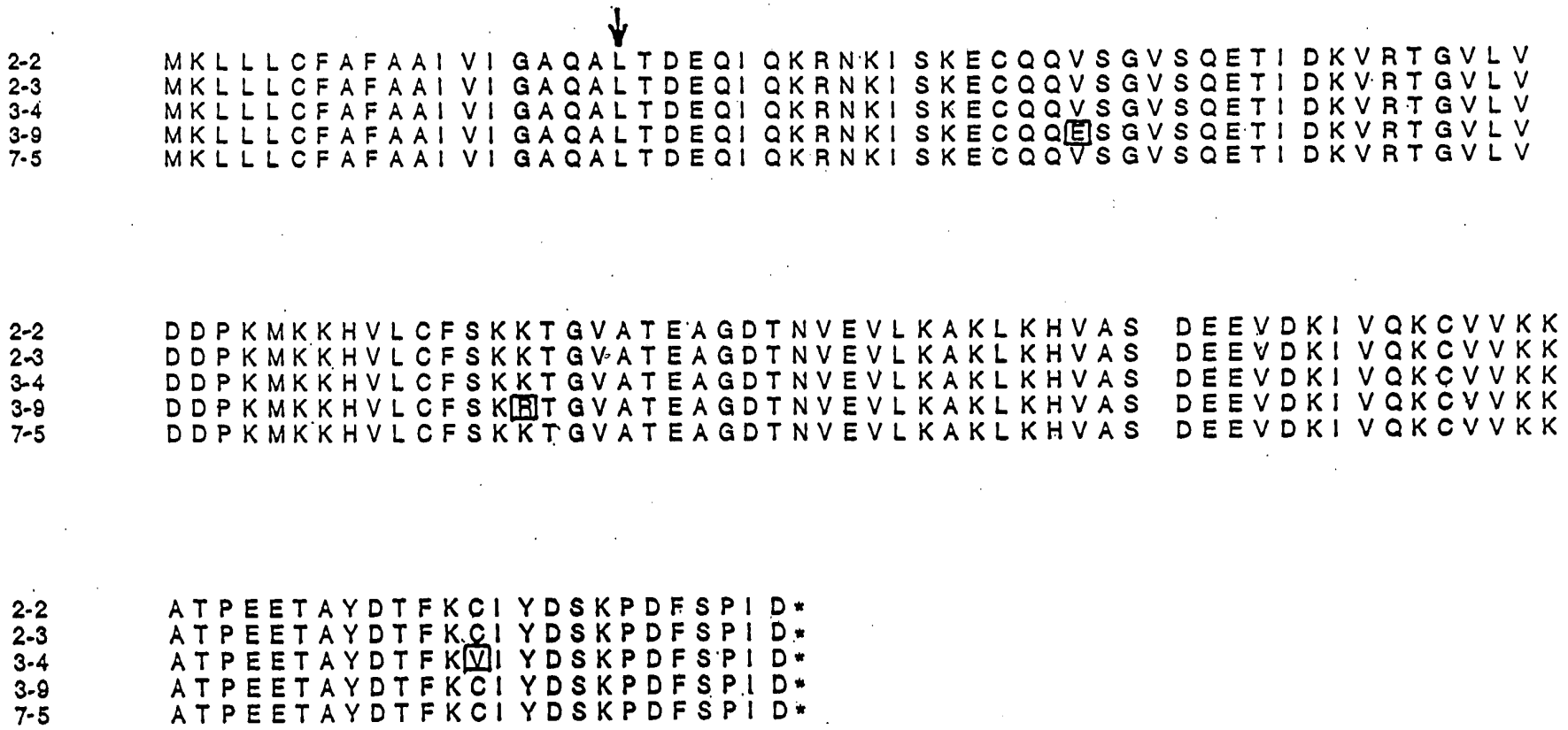

Fig. 4.14

(% mole)

	MW	AA	Cys	Pro	Phe	Ile	Val	Met	Leu	% most hydrophobic	Gly	Ala	Tyr	His	Trp	Asx	Glx	Arg	Lys	Ser	Thr	% most hydrophilic
	(kDa)	(#)																				
Tm 12.86	12.86	117	3.2	3.0	3.4	4.4	8.5	2.0	4.4	28.9	3.1	3.9	3.8	3.2	ND	10.7	15.0	3.6	14.9	6.8	6.3	57.3
Tm 13.17	13.17	116	3.13	2.21	4.47	5.16	10.5	1.0	4.3	29.11	1.73	3.24	0	0	1.41	0	0	7.12	15.6	3.31	6.14	32.14
2-2	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23
2-3	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23
3-4	12.84	115	2.41	3.02	3.44	5.29	11.6	1.02	4.41	28.04	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.09	32.24
3-9	12.87	115	3.21	3.02	3.43	5.28	10.8	1.02	4.40	27.20	1.77	3.31	2.54	2.13	0	0	0	3.64	16.9	4.74	7.07	32.38
7-5	12.84	115	3.21	3.02	3.44	5.29	10.8	1.02	4.41	27.26	1.78	3.32	2.54	2.14	0	0	0	2.43	18.0	4.75	7.08	32.23

Fig. 4.15

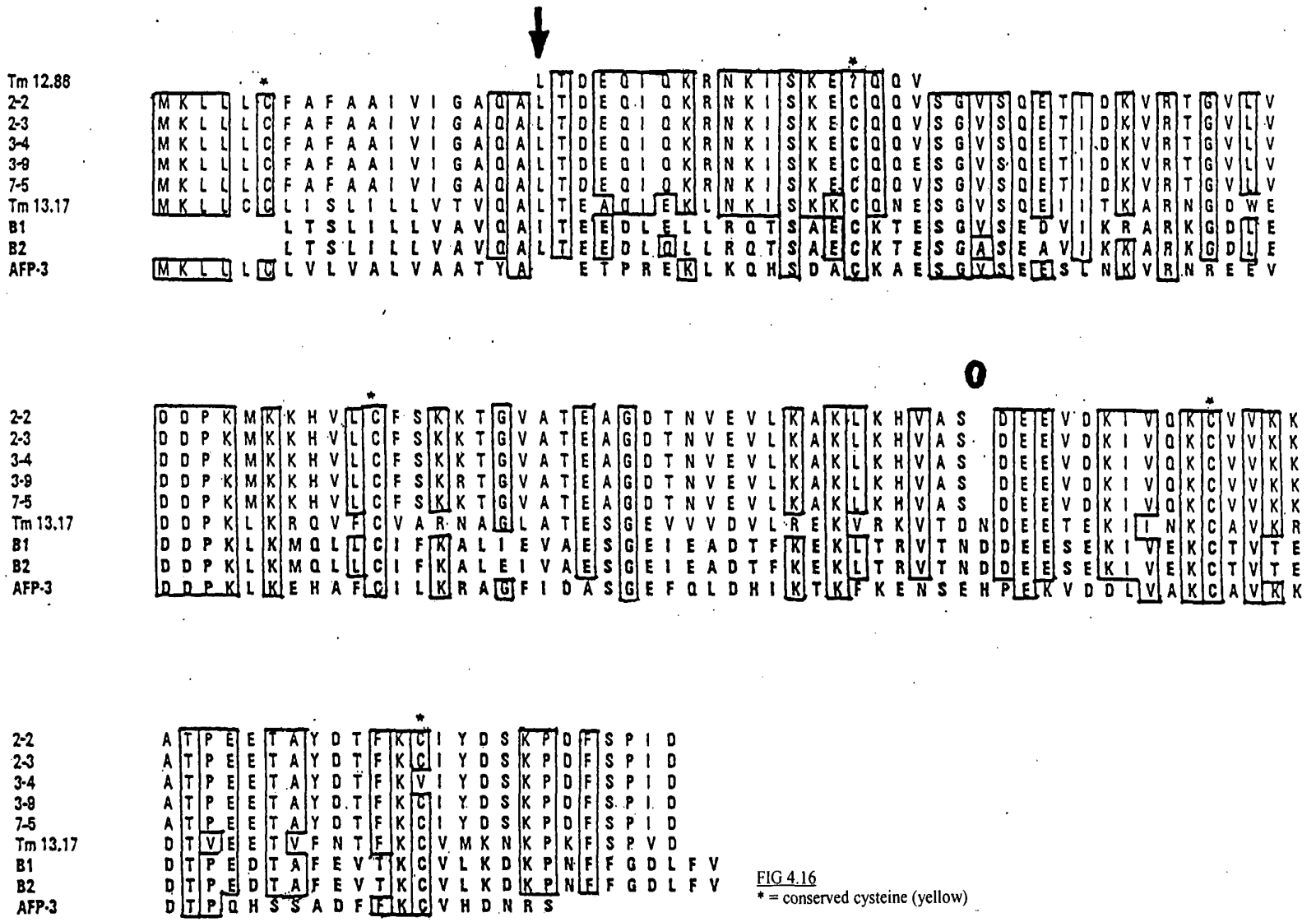

FIG 4.16
* = conserved cysteine (yellow)

Boxed = conserved residues in ≥ 7 Genes (blue or orange)

Fig. 4.16

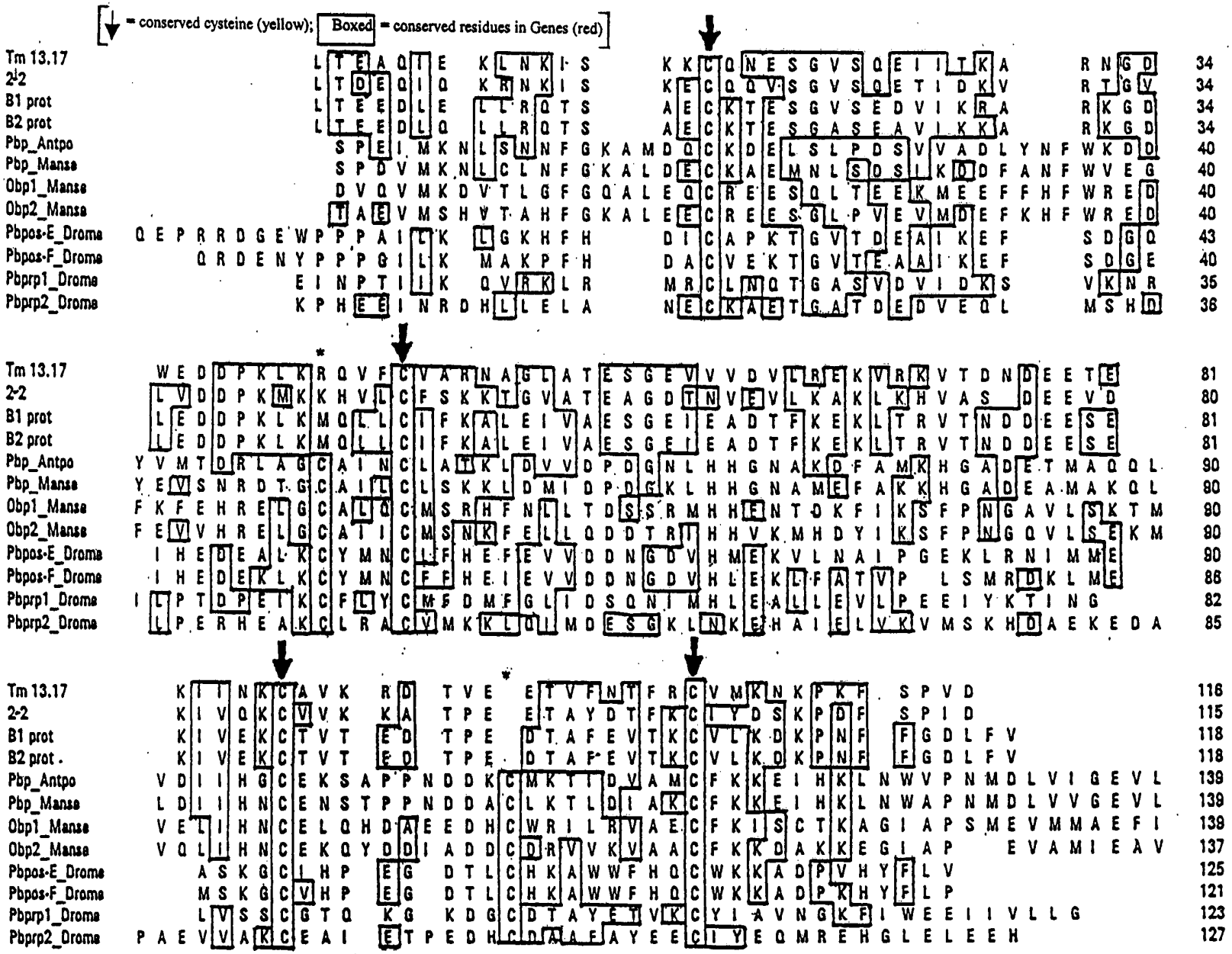
= conserved cysteine (yellow); Boxed = conserved residues in Genes (red)
Tm 13.17
2-2
B1 prot
B2 prot
Pbp_Antpo
Pbp_Manse
Obp1_Manse
Obp2_Manse
Pbpos-E_Drome
Pbpos-F_Drome
Pbprp1_Drome
Pbprp2_Drome

Fig. 4.17

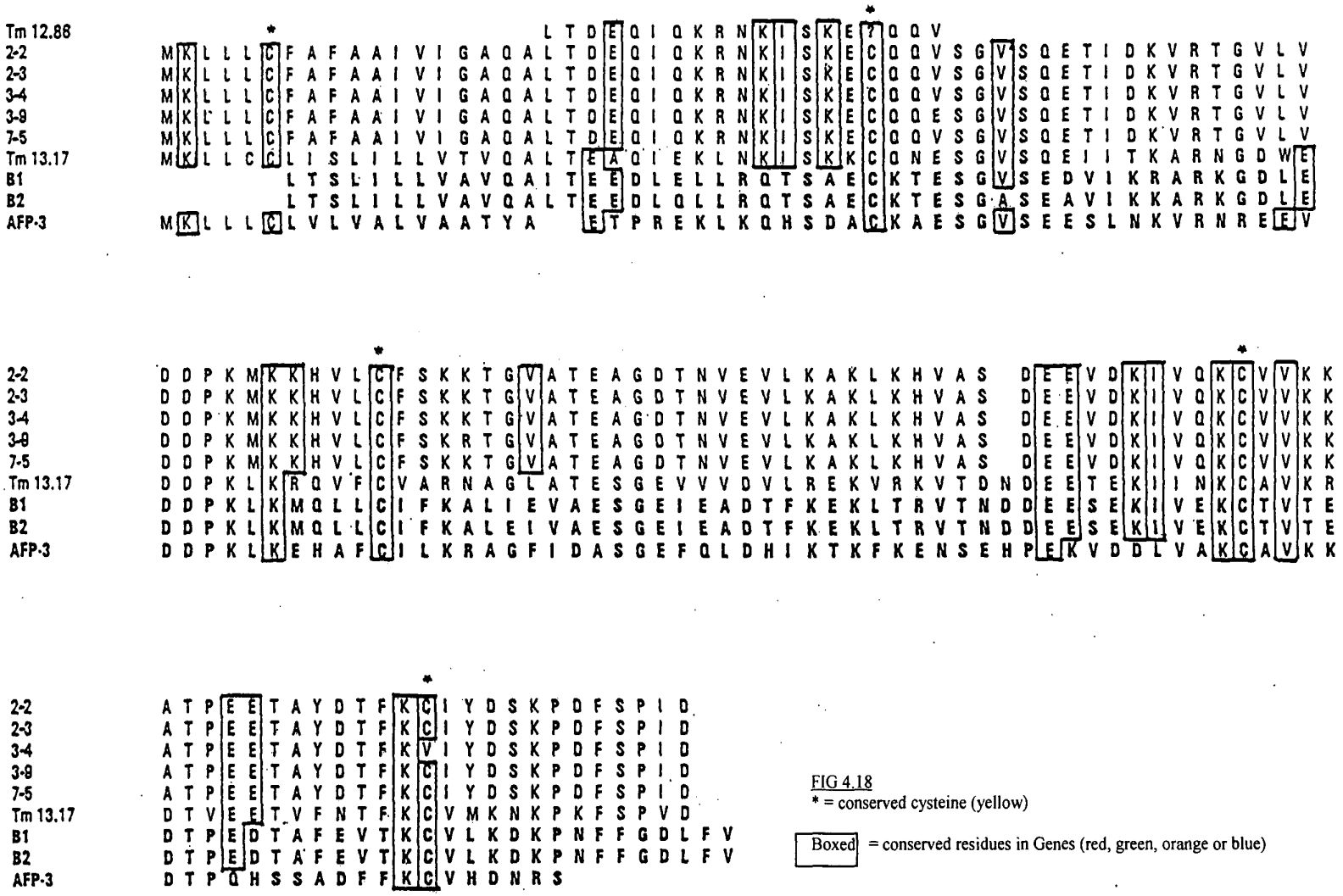

FIG 4.18
* = conserved cysteine (yellow)

Boxed = conserved residues in Genes (red, green, orange or blue)

Fig. 4.18

NUCLEOTIDE SEQUENCES

PERCENT SIMILARITY

PERCENT DIVERGENCE	1 (2-2)	2 (2-3)	3 (3-4)	4 (3-9)	5 (7-5)	6 (Tm13.17)	7 (B1)	8 (AFP-3)	9 (YL-1)	10 (DAFP-1A)		
1		99.1	97.3	98.4	98.2	50.4	42	42.4	20.7	20.9	1	2-2
2	0.9		99	99	99	51.5	43.2	42.7	20.2	21.3	2	2-3
3	1.6	60		98	98	50.3	42	42.1	20	20	3	3-4
4	1.6	20	80		98.4	51.3	43.7	43.6	20.4	22	4	3-9
5	1.8	25	75	45		50.4	42.5	42.9	22.2	23.1	5	7-5
6	36.9	36.4	37.2	36.4	37.1		57.2	37.4	23.3	21	6	Tm 13.17
7	39.9	41.2	41.7	40.7	41.2	30.2		39.3	21.9	22.1	7	B1
8	41.8	42.4	43.1	42.3	42.3	45.3	49.4		21.8	23.1	8	AFP-3
9	48.7	58.6	52	51.2	51.9	61.7	62.2	58.2		45.6	9	YL-1
10	51.4	62.7	46.4	46.5	46.5	60	58.1	61.8	35.5		10	DAFP-1A
	1	2	3	4	5	6	7	8	9	10		

AMINO ACID SEQUENCES

PERCENT SIMILARITY

PERCENT DIVERGENCE	1 (2-2)	2 (2-3)	3 (3-4)	4 (3-9)	5 (7-5)	6 (Tm13.17)	7 (B1)	8 (AFP-3)	9 (YL-1)	10 (DAFP-1A)		
1		100	99.1	99.1	100	51.3	37.4	35.2	11.6	12	1	2-2
2	0		99.1	99.1	100	51.3	37.4	35.2	11.6	12	2	2-3
3	0.9	0.9		98.3	99.1	50.4	36.5	34.3	11.6	12	3	3-4
4	0.9	0.9	1.7		99.1	51.3	37.4	36.1	10.7	12	4	3-9
5	0	0	0.9	0.09		51.3	37.4	35.2	11.6	12	5	7-5
6	46.1	46.1	47	46.1	46.1		47.4	39.8	13.4	13.9	6	Tm 13.17
7	59.1	59.1	60	59.1	59.1	51.7		37	11.6	11.1	7	B1
8	61.7	61.7	62.6	60.7	61.7	60.2	63		10.2	8.3	8	AFP-3
9	86.7	86.7	85.7	85.7	85.7	86.8	84.2	87.5		55.6	9	YL-1
10	88.3	88.3	86.4	84.5	85.4	89.1	82.7	90.5	40.2		10	DAFP-1A
	1	2	3	4	5	6	7	8	9	10		

Fig. 4.19

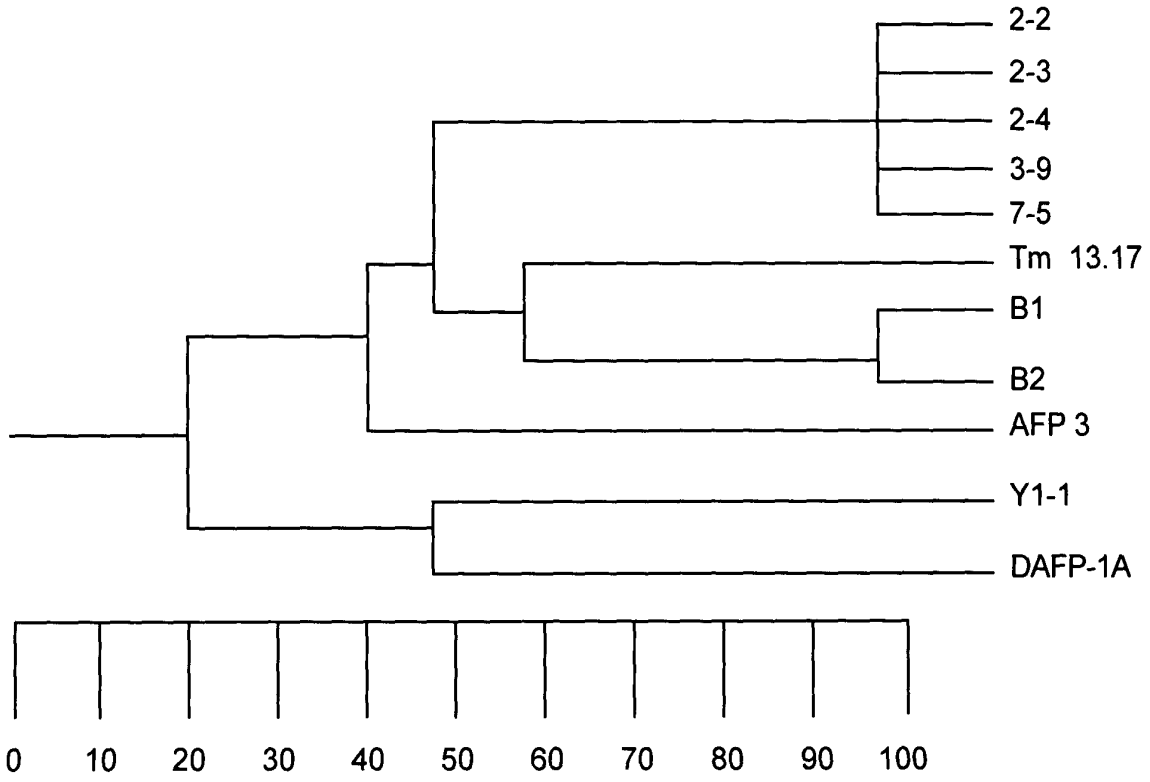
2-2
2-3
2-4
3-9
7-5
Tm 13.17
B1
B2
AFP 3
Y1-1
DAFP-1A
0
10
20
30
40
50
60
70
80
90
100
% Nucleic Acid Identity

Fig. 4.20

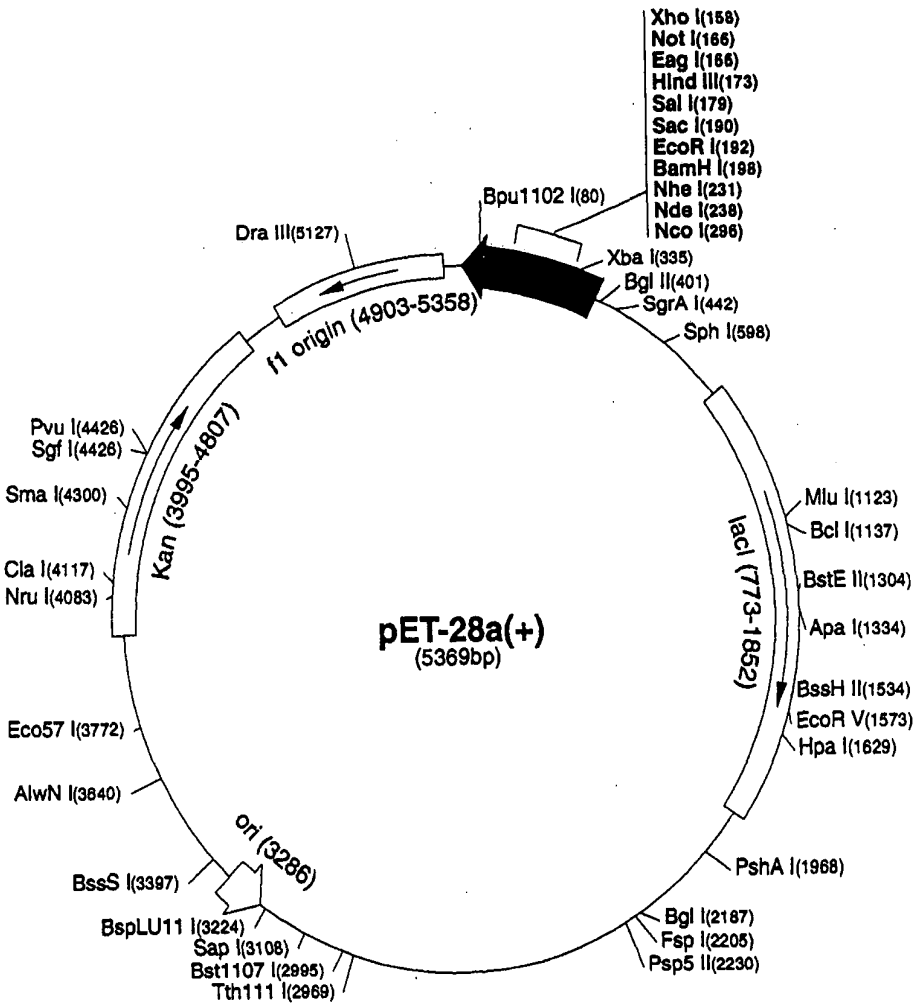
Xho I(158)
Not I(166)
Eag I(166)
Hind III(173)
Sal I(179)
Sac I(190)
EcoR I(192)
BamH I(198)
Nhe I(231)
Nde I(238)
Nco I(296)
Bpu1102 I(80)
Dra III(5127)
Xba I(335)
Bgl II(401)
SgrA I(442)
Sph I(598)
f1 origin (4903-5358)
Pvu I(4426)
Sgf I(4426)
Sma I(4300)
Kan (3995-4807)
Cla I(4117)
Nru I(4083)
Mlu I(1123)
Bcl I(1137)
BstE II(1304)
Apa I(1334)
lacI (773-1852)
pET-28a(+)
(5369bp)
BssH II(1534)
EcoR V(1573)
Hpa I(1629)
Eco57 I(3772)
AlwN I(3640)
ori (3286)
PshA I(1968)
BssS I(3397)
Bgl I(2187)
Fsp I(2205)
BspLU11 I(3224)
Sap I(3108)
Psp5 II(2230)
Bst1107 I(2995)
Tth111 I(2969)

Fig. 5.0

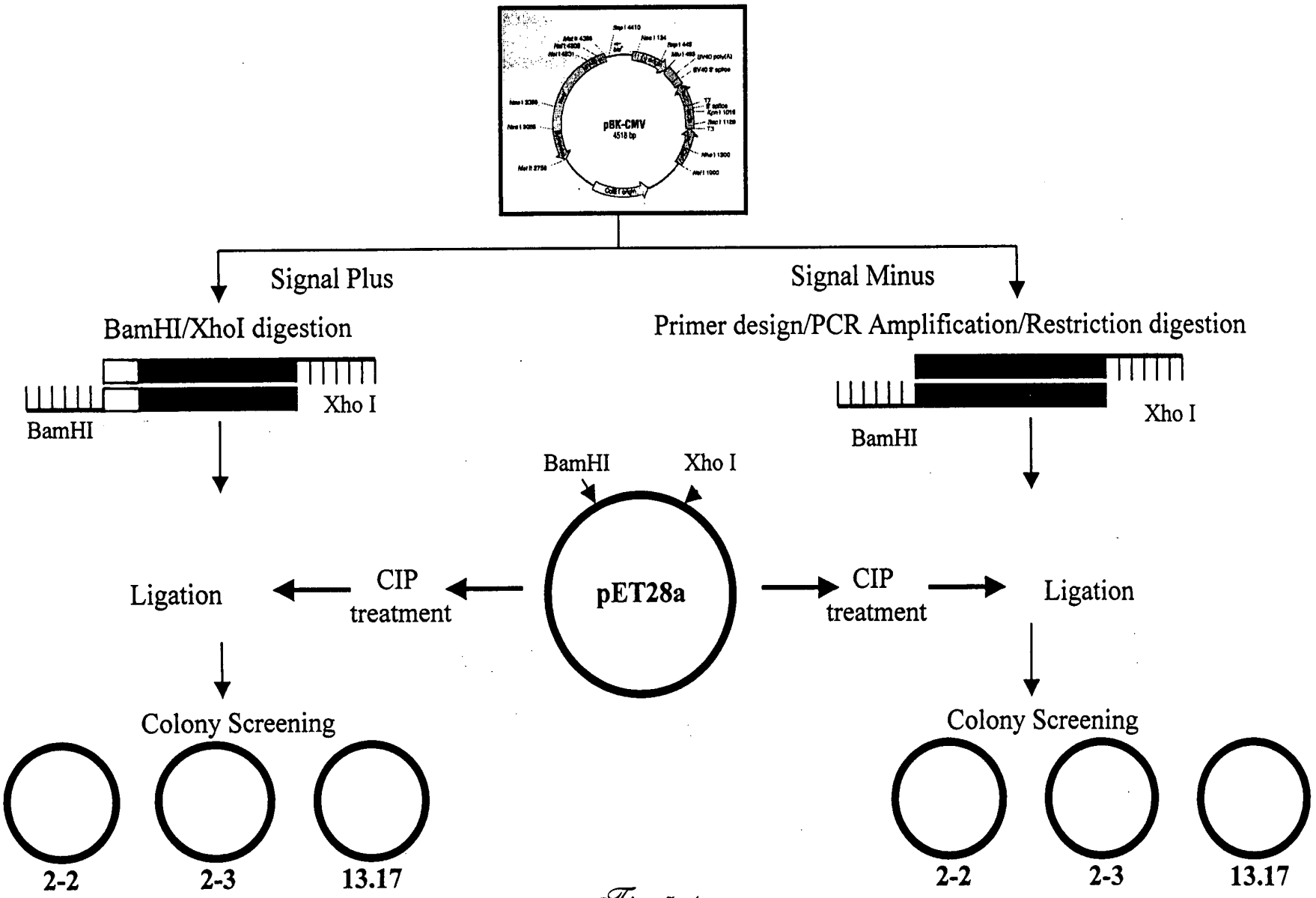
pBK-CMV
4518 bp
Signal Plus
BamHI/XhoI digestion
BamHI
Xho I
Signal Minus
Primer design/PCR Amplification/Restriction digestion
BamHI
Xho I
BamHI
Xho I
pET28a
Ligation
CIP treatment
CIP treatment
Ligation
Colony Screening
Colony Screening
2-2
2-3
13.17
2-2
2-3
13.17

Fig. 5. 1

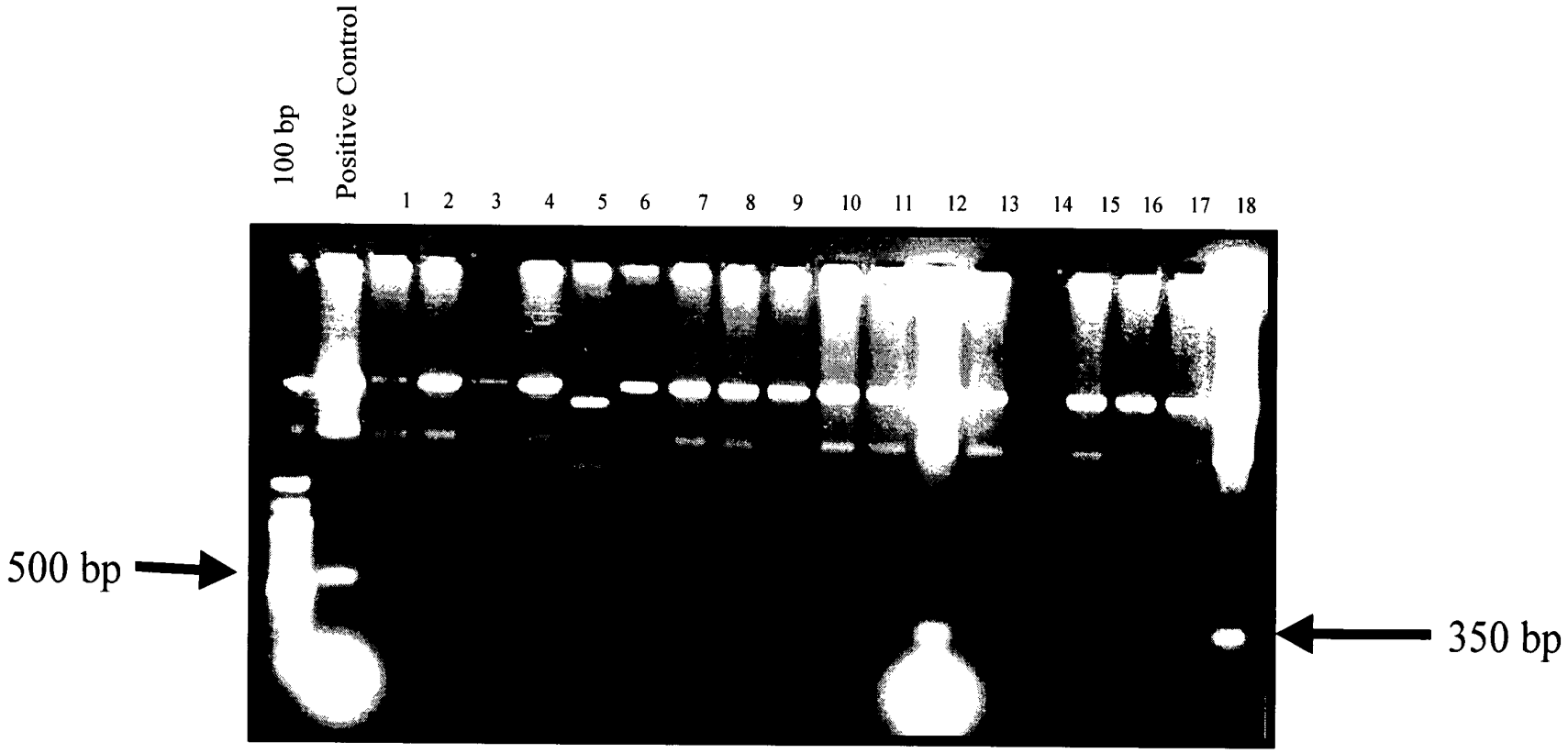
100 bp
Positive Control
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
500 bp
350 bp

Fig. 5.2

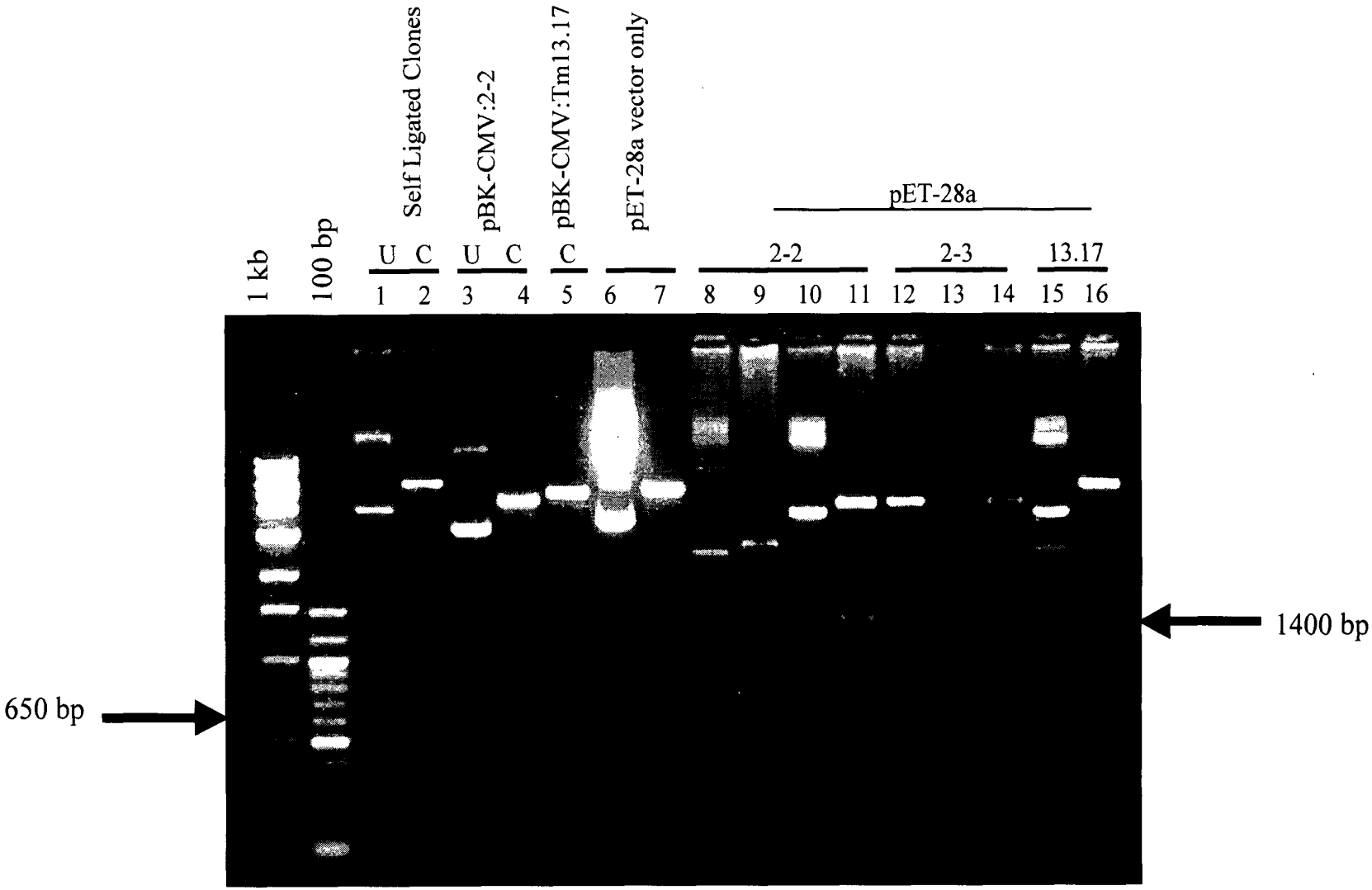
1 kb
100 bp
Self Ligated Clones
U C
pBK-CMV:2-2
U C
pBK-CMV:Tm13.17
C
pET-28a vector only
pET-28a
2-2
2-3
13.17
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
650 bp
1400 bp

Fig. 5.3

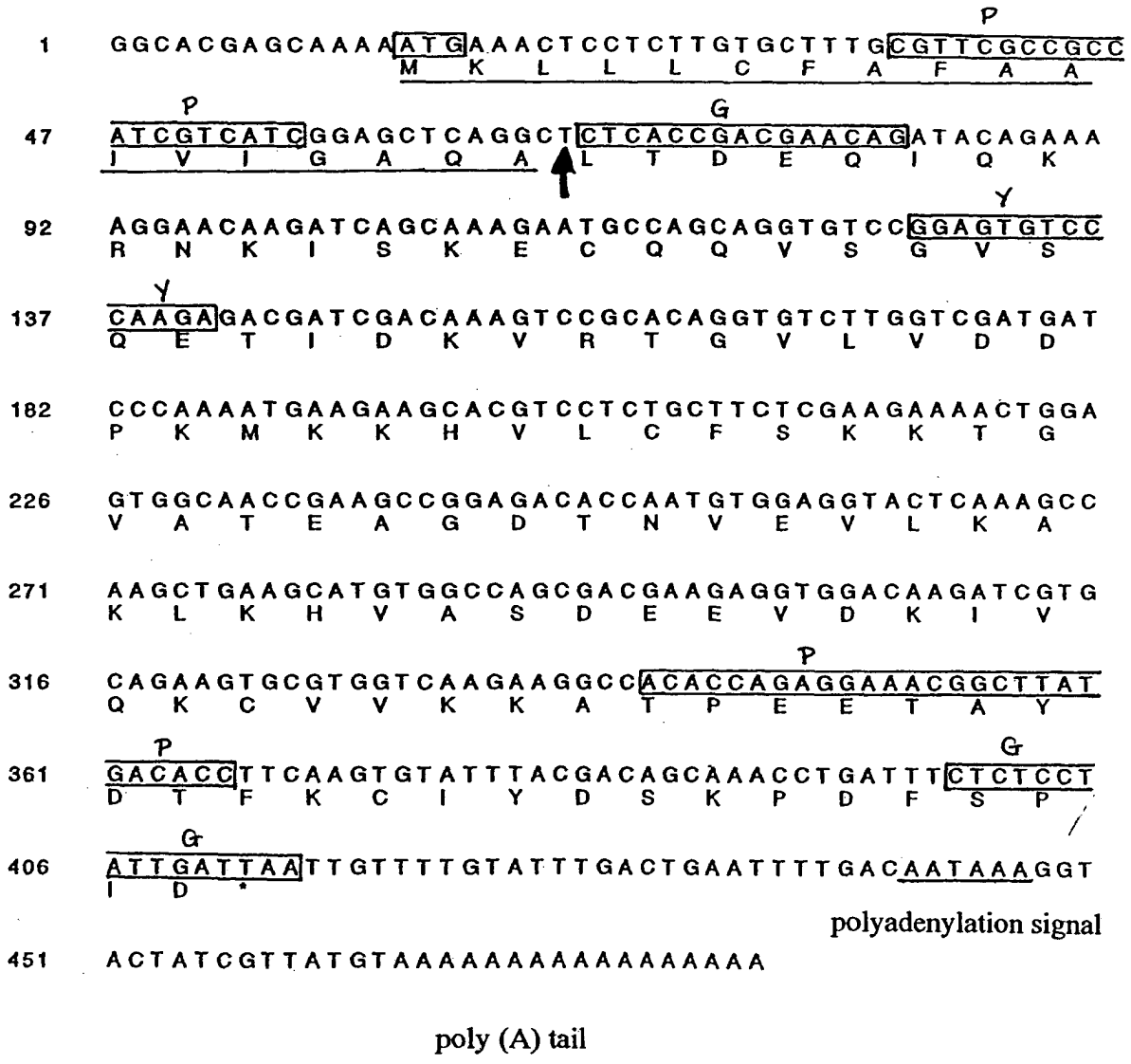
1 GGCACGAGCAAAA ATG AAACTCCTCTTGTGCTTTG CGTTCGCCGCC
M K L L L C F A F A A
47 ATCGTCATC GGAGCTCAGGCT CTCACCGACGAACAG ATACAGAAA
I V I G A Q A L T D E Q I Q K
92 AGGAACAAGATCAGCAAAGAATGCCAGCAGGTGTCC GGAGTGTCC
R N K I S K E C Q Q V S G V S
137 CAAGA GACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGATGAT
Q E T I D K V R T G V L V D D
182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACTGGA
P K M K K H V L C F S K K T G
226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC
V A T E A G D T N V E V L K A
271 AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG
K L K H V A S D E E V D K I V
316 CAGAAGTGCGTGGTCAAGAAGGCC ACACCAGAGGAAACGGCTTAT
Q K C V V K K A T P E E T A Y
361 GACACC TTCAAGTGTATTTACGACAGCAAACCTGATTT CTCTCCT
D T F K C I Y D S K P D F S P
406 ATTGATTAA TTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT
I D *
polyadenylation signal
451 ACTATCGTTATGTAAAAAAAAAAAAAAAAA
poly (A) tail

Fig. 8.44

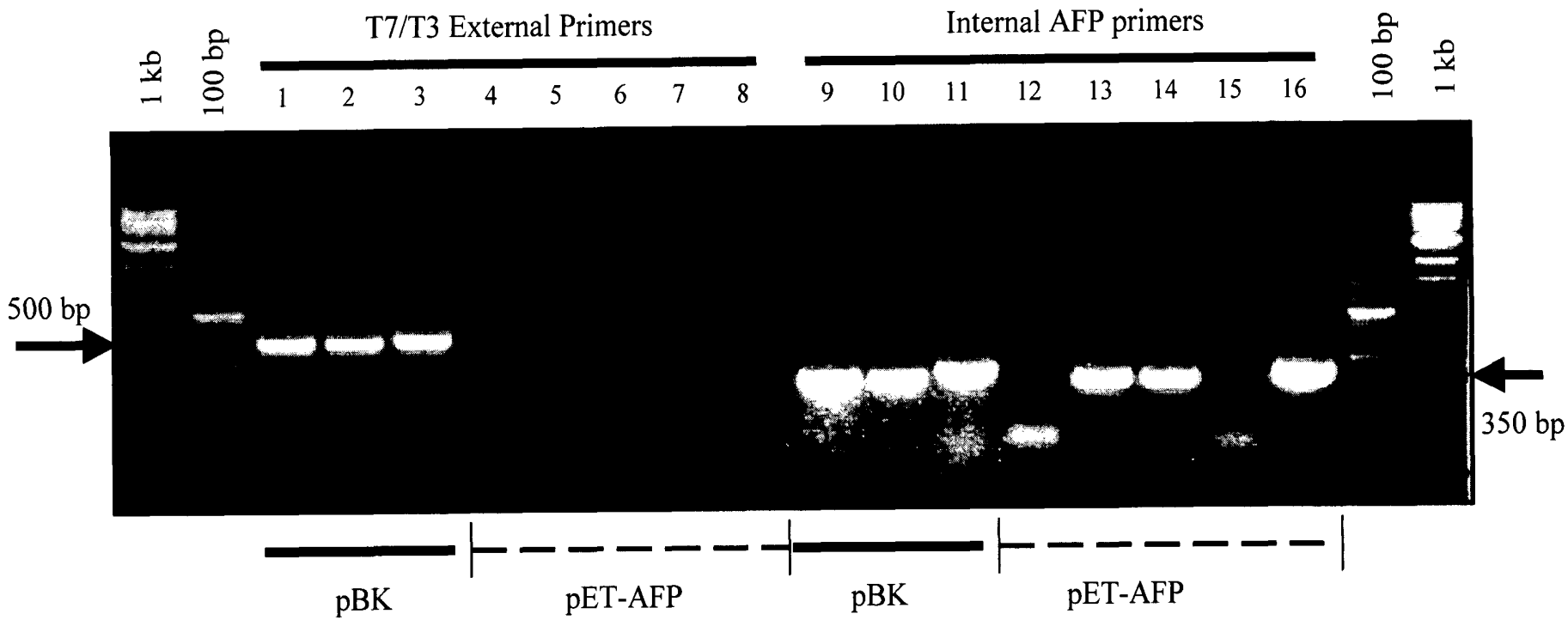
1 kb
100 bp
T7/T3 External Primers
1
2
3
4
5
6
7
8
Internal AFP primers
9
10
11
12
13
14
15
16
100 bp
1 kb
500 bp
350 bp
pBK
pET-AFP
pBK
pET-AFP

Fig. 5.4

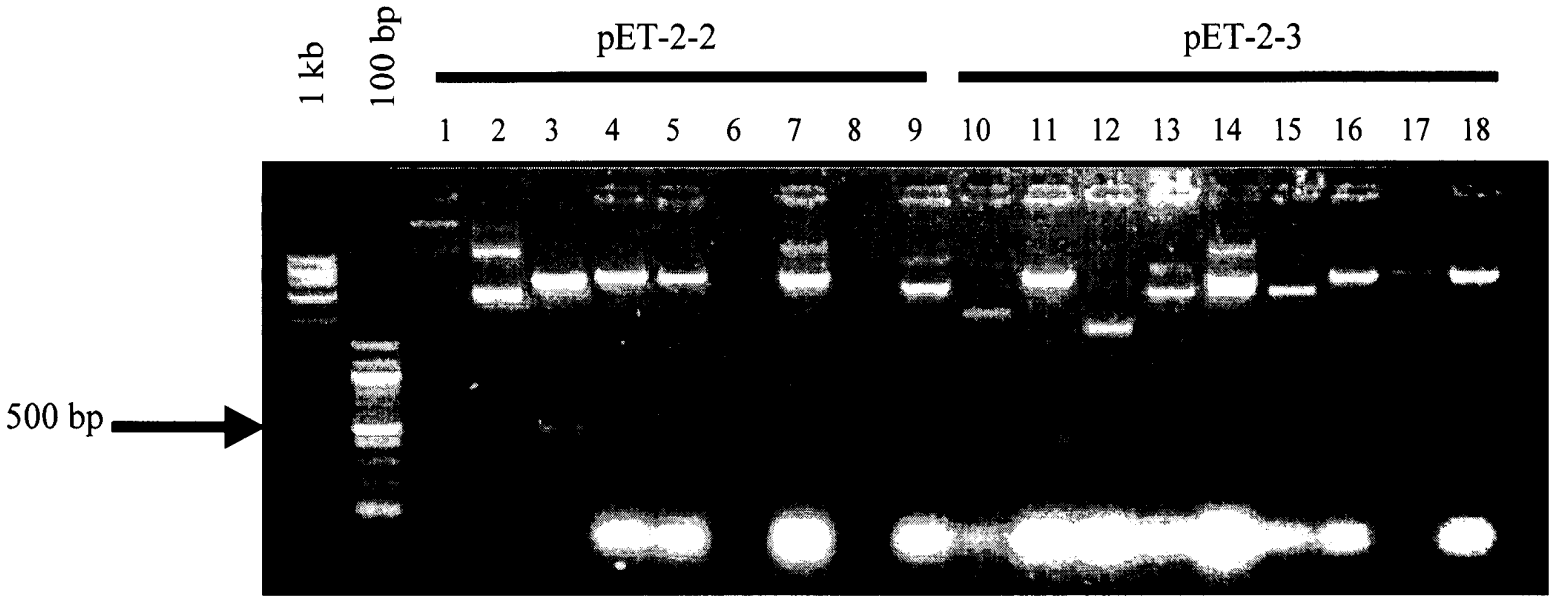
1 kb
100 bp
pET-2-2
pET-2-3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
500 bp

Fig. 5.5

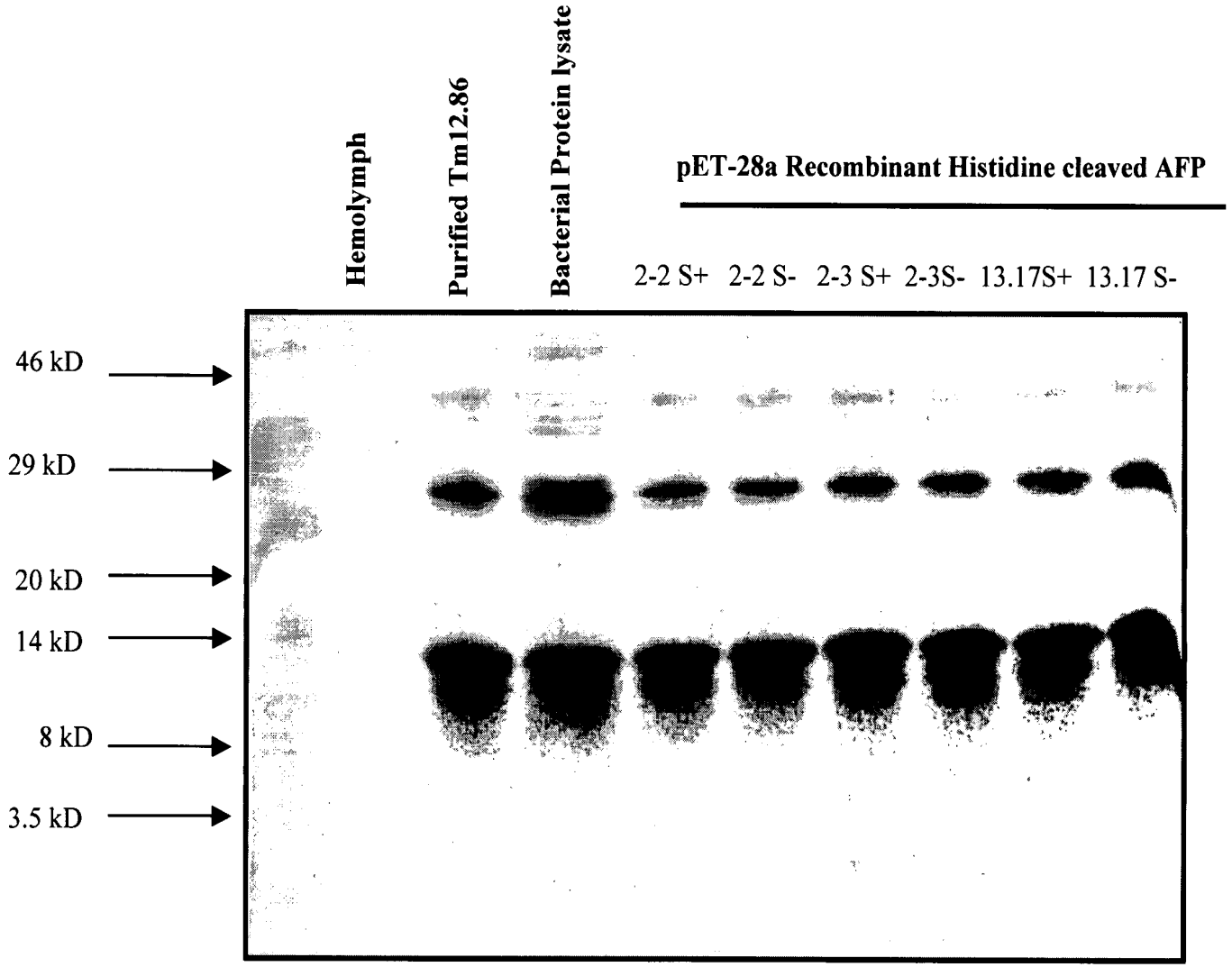
Hemolymph
Purified Tm12.86
Bacterial Protein lysate
pET-28a Recombinant Histidine cleaved AFP
2-2 S+
2-2 S-
2-3 S+
2-3S-
13.17S+
13.17 S-
46 kD
29 kD
20 kD
14 kD
8 kD
3.5 kD

Fig 5.6

His-tagged Clone 2.2 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                           -55                 -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
       -45                 -40                 -35

                                                        AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG             186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
       -30                 -25                 -20

AAA CTC CTC TTG TGC TTT GCG TTC GCC GCC ATC GTC ATC GGA GCT             231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
       -15                 -10                 -5

       N-terminal of mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC             276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
       1               5                   10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC             321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG             366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC             411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG             456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAG GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC             501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT             546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                                Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA          595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTAATA TCGTTATGTA AAAAAAAAAA                  645

AAAAAACTCG AGCACCACCA CCACCACCAC TGAGAT                                 681
```

Fig. 5.7

His-tagged clone 2.2 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG          50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC      96
               Met Gly Ser Ser His His His His His His Ser
                               -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT   141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
           -20                 -15                 -10

                                   N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG   186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
           -5                  1                   5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG   231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
       10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT   276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
       25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT   321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
       40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA   366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
       55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAG GTG GAC AAG ATC   411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
       70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT   456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
       85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT   501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
       100                 105                 110

           Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT              543
Pro Ile Asp *
       115
```

Fig. 5.8

His-tagged clone 2.3 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC             96
                Met Gly Ser Ser His His His His His His Ser
                           -55                 -50

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT           141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
        -45                 -40                 -35

                                                        AFP Start Codon
GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC GCA CGA GCA AAA ATG           186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Ala Arg Ala Lys Met
        -30                 -25                 -20

AAA CTC CTC TTG TGC TTT GCT TTC GCC GCC ATC GTC ATC GGA GCT           231
Lys Leu Leu Leu Cys Phe Ala Phe Ala Ala Ile Val Ile Gly Ala
        -15                 -10                 -5

        N-terminal of Mature AFP
CAG GCT CTC ACC GAC GAA CAG ATA CAG AAA AGG AAC AAG ATC AGC           276
Gln Ala Leu Thr Asp Glu Gln Ile Gln Lys Arg Asn Lys Ile Ser
        1               5                   10

AAA GAA TGC CAG CAG GTG TCC GGA GTG TCC CAA GAG ACG ATC GAC           321
Lys Glu Cys Gln Gln Val Ser Gly Val Ser Gln Glu Thr Ile Asp
    15                  20                  25

AAA GTC CGC ACA GGT GTC TTG GTC GAT GAT CCC AAA ATG AAG AAG           366
Lys Val Arg Thr Gly Val Leu Val Asp Asp Pro Lys Met Lys Lys
    30                  35                  40

CAC GTC CTC TGC TTC TCG AAG AAA ACT GGA GTG GCA ACC GAA GCC           411
His Val Leu Cys Phe Ser Lys Lys Thr Gly Val Ala Thr Glu Ala
    45                  50                  55

GGA GAC ACC AAT GTG GAG GTA CTC AAA GCC AAG CTG AAG CAT GTG           456
Gly Asp Thr Asn Val Glu Val Leu Lys Ala Lys Leu Lys His Val
    60                  65                  70

GCC AGC GAC GAA GAA GTG GAC AAG ATC GTG CAG AAG TGC GTG GTC           501
Ala Ser Asp Glu Glu Val Asp Lys Ile Val Gln Lys Cys Val Val
    75                  80                  85

AAG AAG GCC ACA CCA GAG GAA ACG GCT TAT GAC ACC TTC AAG TGT           546
Lys Lys Ala Thr Pro Glu Glu Thr Ala Tyr Asp Thr Phe Lys Cys
    90                  95                  100

                                                Stop Codon
ATT TAC GAC AGT AAA CCT GAT TTC TCT CCT ATT GAT TAA TTGTTTTGTA        595
Ile Tyr Asp Ser Lys Pro Asp Phe Ser Pro Ile Asp *
    105                 110                 115

                Polyadenylation signal    Poly-A tail
TTTGACTGAA TTTTGACAAT AAAGGTACTA TCGTTATGAA AAAAAAAAAA               645

AAAAAAACTC GAGCACCACC ACCACCACCA CTGAGAT                             682
```

Fig. 5.9

His-tagged Clone 2.3 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG          50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC      96
               Met Gly Ser Ser His His His His His His Ser
                                -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT    141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
            -20                 -15                 -10

                                    N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGA TCC CTC ACC GAC GAA CAG ATA CAG    186
Gly Gln Gln Met Gly Arg Gly Ser Leu Thr Asp Glu Gln Ile Gln
            -5                  1               5

AAA AGG AAC AAG ATC AGC AAA GAA TGC CAG CAG GTG TCC GGA GTG    231
Lys Arg Asn Lys Ile Ser Lys Glu Cys Gln Gln Val Ser Gly Val
        10                  15                  20

TCC CAA GAG ACG ATC GAC AAA GTC CGC ACA GGT GTC TTG GTC GAT    276
Ser Gln Glu Thr Ile Asp Lys Val Arg Thr Gly Val Leu Val Asp
        25                  30                  35

GAT CCC AAA ATG AAG AAG CAC GTC CTC TGC TTC TCG AAG AAA ACT    321
Asp Pro Lys Met Lys Lys His Val Leu Cys Phe Ser Lys Lys Thr
        40                  45                  50

GGA GTG GCA ACC GAA GCC GGA GAC ACC AAT GTG GAG GTA CTC AAA    366
Gly Val Ala Thr Glu Ala Gly Asp Thr Asn Val Glu Val Leu Lys
        55                  60                  65

GCC AAG CTG AAG CAT GTG GCC AGC GAC GAA GAA GTG GAC AAG ATC    411
Ala Lys Leu Lys His Val Ala Ser Asp Glu Glu Val Asp Lys Ile
        70                  75                  80

GTG CAG AAG TGC GTG GTC AAG AAG GCC ACA CCA GAG GAA ACG GCT    456
Val Gln Lys Cys Val Val Lys Lys Ala Thr Pro Glu Glu Thr Ala
        85                  90                  95

TAT GAC ACC TTC AAG TGT ATT TAC GAC AGT AAA CCT GAT TTC TCT    501
Tyr Asp Thr Phe Lys Cys Ile Tyr Asp Ser Lys Pro Asp Phe Ser
        100                 105                 110

            Stop Codon
CCT ATT GAT TAA CTCGAGCACC ACCACCACCA CCACTGAGAT               543
Pro Ile Asp *
        115
```

Fig. 5.10

His-tagged Tm 13.17 with signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
               -65                 -60                 -55

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT             141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
               -50                 -45                 -40

GGA CAG CAA ATG GGT CGC GGA TCC GAA TTC TGG ATC CAA AGA ATT             186
Gly Gln Gln Met Gly Arg Gly Ser Glu Phe Trp Ile Gln Arg Ile
               -35                 -30                 -25

                        AFP Start Codon
CGG CAC GAG ACT ACT AAG ATG AAG TTG CTC TGT TGT CTA ATC TCC             231
Arg His Glu Thr Thr Lys Met Lys Leu Leu Cys Cys Leu Ile Ser
               -20                 -15                 -10

                                    N-terminal of mature AFP
CTC ATT CTG TTG GTC ACA GTT CAG GCC CTG ACC GAG GCA CAA ATT             276
Leu Ile Leu Leu Val Thr Val Gln Ala Leu Thr Glu Ala Gln Ile
               -5                  1                   5

GAG AAA CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA             321
Glu Lys Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly
           10                  15                  20

GTG TCG CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG             366
Val Ser Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu
           25                  30                  35

GAC GAT CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC             411
Asp Asp Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn
           40                  45                  50

GCC GGT CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG             456
Ala Gly Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu
           55                  60                  65

AGG GAG AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG             501
Arg Glu Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu
           70                  75                  80

AAA ATC ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG             546
Lys Ile Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu
           85                  90                  95

ACG GTG TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG             595
Thr Val Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys
           100                 105                 110

                    Stop Codon
TTC TCA CCA GTT GAT TGA ACCACCACGA CTAGTAGATG GTTCAAATGG                643
Phe Ser Pro Val Asp *
           115

                  Polyadenylation signal  Poly-A tail
TGTGCTTTAC ATATAAAAAT AAAGTGTTTC TGATGTAAAA AAAAAAAAAA                  693

AAAAAAAAAA AACTCGAGAG TATTCTAGAG CGGCCGCGGG CCCATCGTTT                  743

TCCACCCCTC GAGCACCACC ACCACCACCA CTGAGAT                                777
```

Fig. 5.11

His-tagged Tm 13.17 without signal sequence

```
TTGTTAGCGG ATGGAATTCC CTCGTAGGGG ATAATTTTGT TTACTTTAAG                  50

                His-tag Start Codon
AAGGAGATAT ACC ATG GGC AGC AGC CAT CAT CAT CAT CAT CAC AGC               96
               Met Gly Ser Ser His His His His His His Ser
                           -30                 -25

AGC GGC CTG GTG CCG CGC GGC AGC CAT ATG GCT AGC ATG ACT GGT              141
Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly
        -20                 -15                 -10

                                N-terminal of mature AFP
GGA CAG CAA ATG GGT CGC GGC CTG ACC GAG GCA CAA ATT GAG AAA              186
Gly Gln Gln Met Gly Arg Gly Leu Thr Glu Ala Gln Ile Glu Lys
        -5                  1               5

CTG AAC AAG ATC AGC AAA AAA TGT CAA AAT GAA AGT GGA GTG TCG              231
Leu Asn Lys Ile Ser Lys Lys Cys Gln Asn Glu Ser Gly Val Ser
    10                  15                  20

CAA GAG ATC ATA ACC AAA GCT CGC AAC GGT GAC TGG GAG GAC GAT              276
Gln Glu Ile Ile Thr Lys Ala Arg Asn Gly Asp Trp Glu Asp Asp
    25                  30                  35

CCT AAA CTG AAA CGC CAA GTT TTT TGC GTG GCC AGG AAC GCC GGT              321
Pro Lys Leu Lys Arg Gln Val Phe Cys Val Ala Arg Asn Ala Gly
    40                  45                  50

CTG GCC ACG GAA TCG GGA GAG GTG GTG GTC GAC GTG TTG AGG GAG              366
Leu Ala Thr Glu Ser Gly Glu Val Val Val Asp Val Leu Arg Glu
    55                  60                  65

AAG GTG AGG AAG GTC ACT GAC AAC GAC GAA GAA ACT GAG AAA ATC              411
Lys Val Arg Lys Val Thr Asp Asn Asp Glu Glu Thr Glu Lys Ile
    70                  75                  80

ATC AAT AAG TGC GCC GTC AAG AGA GAT ACT GTT GAA GAG ACG GTG              456
Ile Asn Lys Cys Ala Val Lys Arg Asp Thr Val Glu Glu Thr Val
    85                  90                  95

TTC AAT ACT TTC AAA TGT GTC ATG AAA AAC AAG CCA AAG TTC TCA              501
Phe Asn Thr Phe Lys Cys Val Met Lys Asn Lys Pro Lys Phe Ser
    100                 105                 110

          Stop Codon
CCA GTT GAT TGA CTCGAGCACC ACCACCACCA CCACTGAGAT                        543
Pro Val Asp *
    115
```

Fig. 5.12

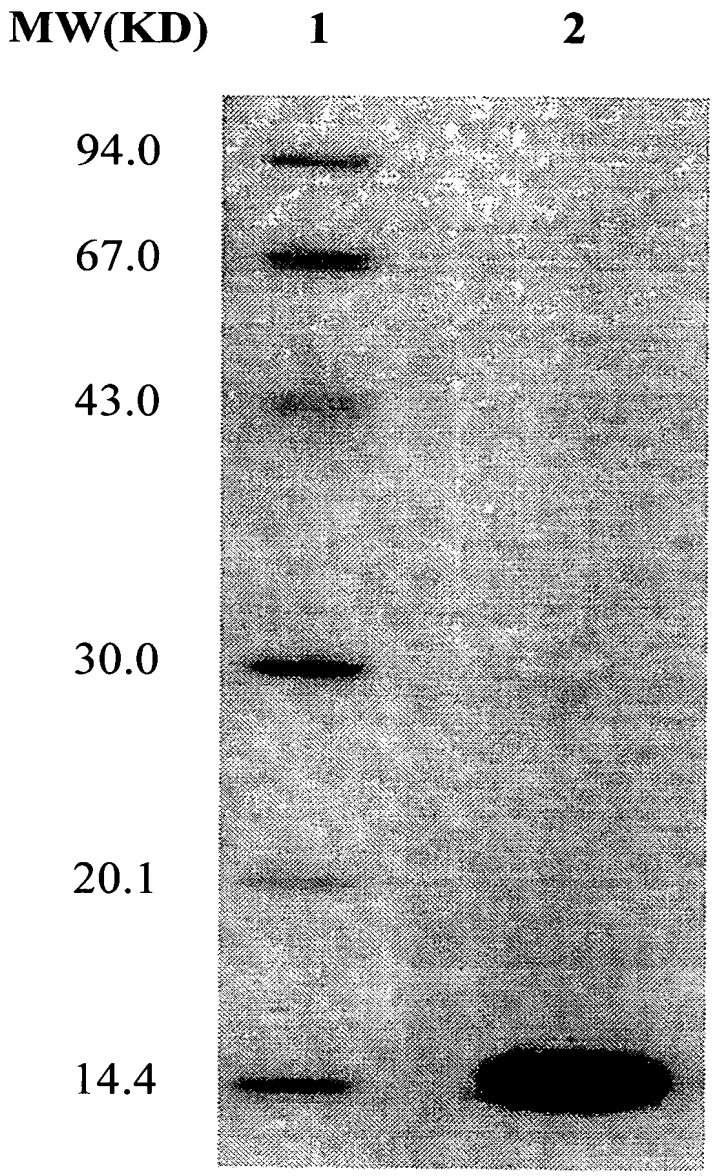

Fig. 6.0

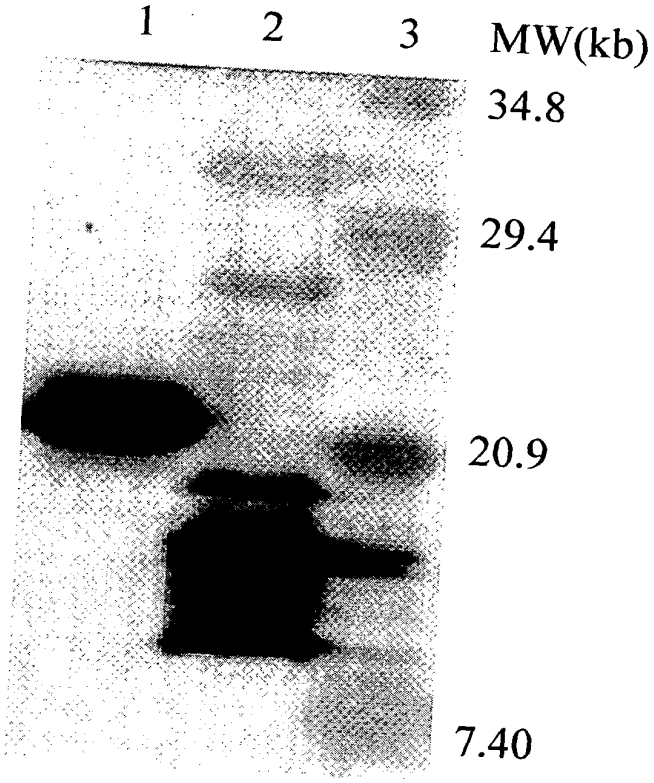

Fig. 6.1

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Fig. 6.2

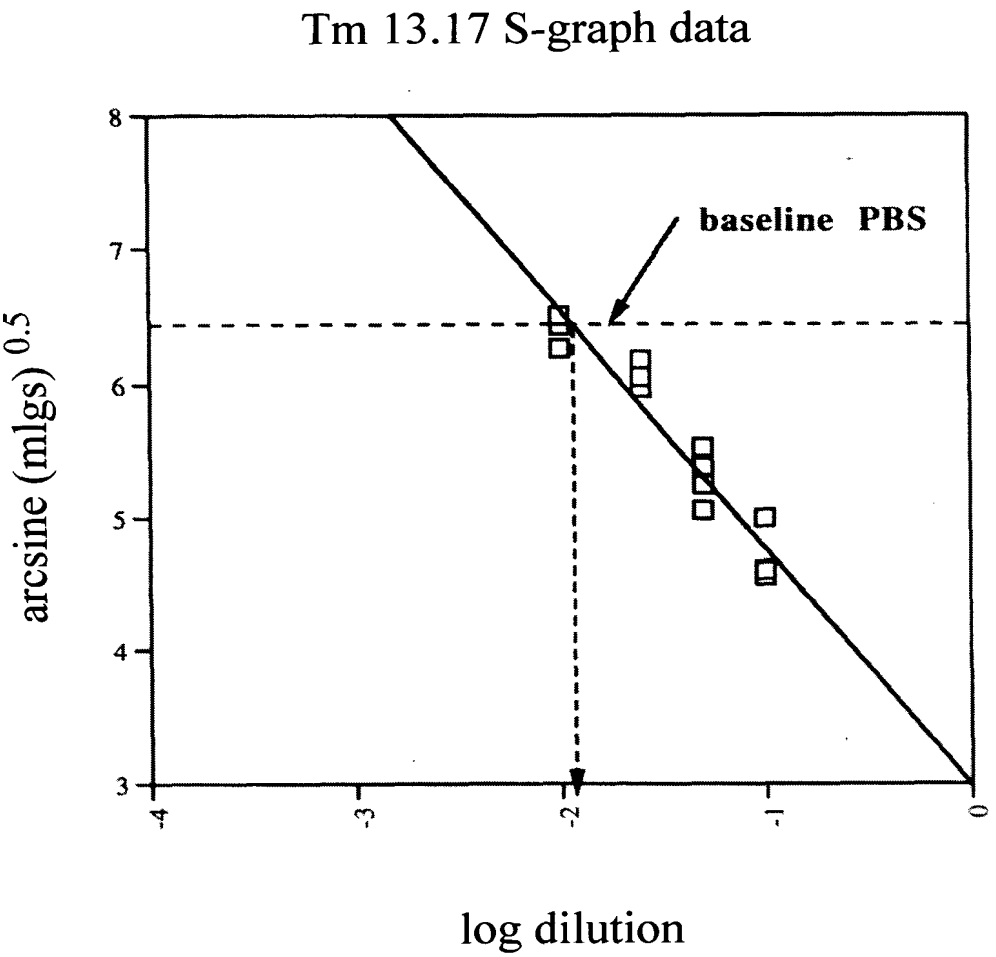

Tm 13.17 S-graph data
baseline PBS
arcsine (mlgs) 0.5
8
7
6
5
4
3
-4
-3
-2
-1
0
log dilution

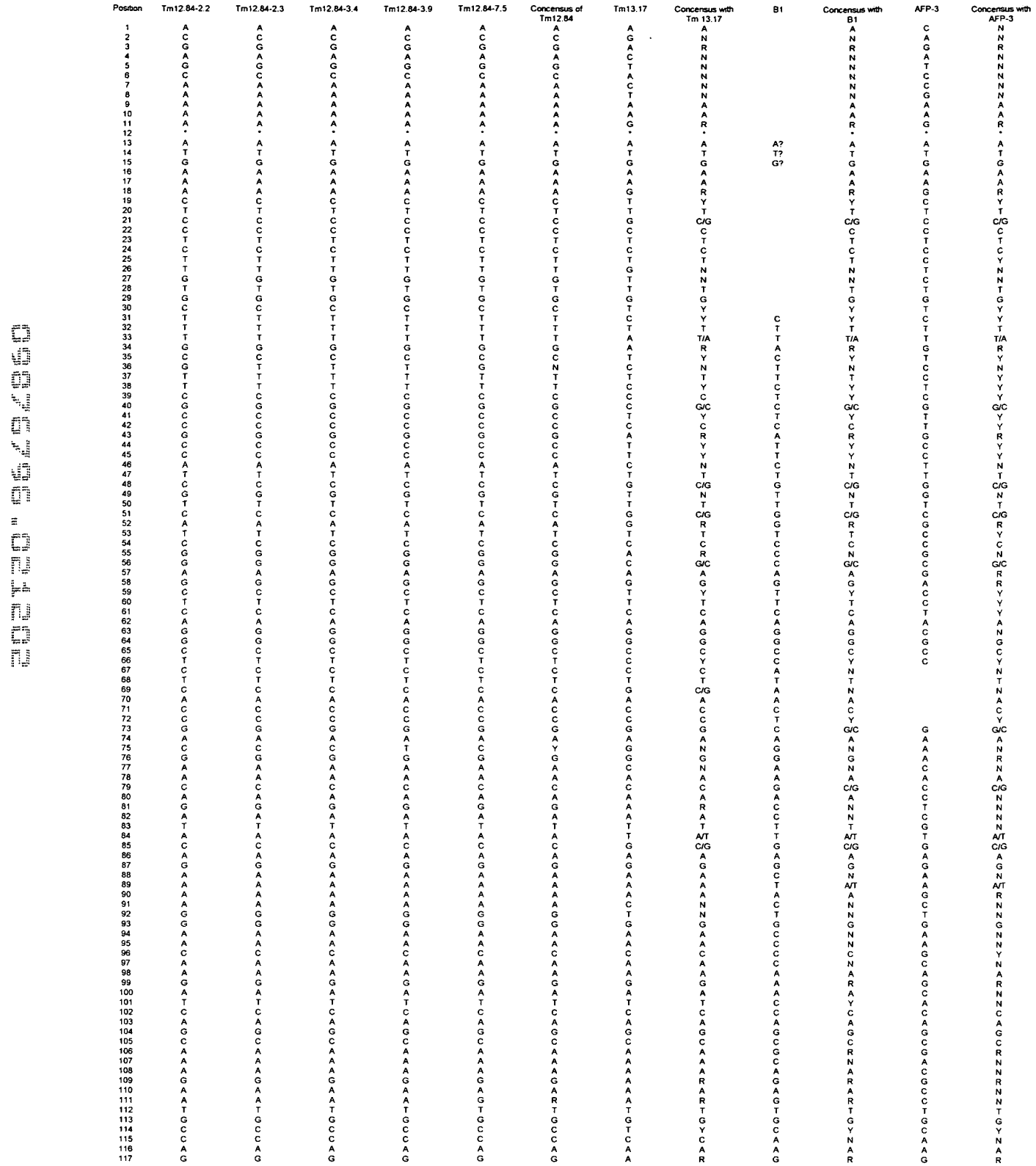
Fig. 6.3

ONE LETTER	NAME	THREE LETTER	CHEMICAL CLASS	HYDROPHOBICITY	Reactivity
A	Alanine	Ala	aliphatic	mod. hydrophobic	low
B	Asp or Asn	Asx			
C	Cysteine	Cys	sulfhydryl	hydrophobic	high
D	Aspartic Acid	Asp	acidic	highly hydrophilic	high
E	Glutamic Acid	Glu	acidic	highly hydrophilic	high
F	Phenylalanine	Phe	aromatic	highly hydrophobic	low
G	Glycine	Gly	aliphatic	mod. hydrophobic	low
H	Histidine	His	basic, imidazole	highly hydrophilic	high
I	Isoleucine	Ile	aliphatic	hydrophobic	low
J					
K	Lysine	Lys	basic	highly hydophilic	high
L	Leucine	Leu	aliphatic	hydrophobic	low
M	Methionine	Met	sulfhydryl	hydrophobic	low
N	Asparagine	Asn	amide, acidic derived	hydrophilic	high
O					
P	Proline	Pro	aliphatic, cyclic, imino	mod. hydrophilic	low
Q	Glutamine	Gln	amide, acidic derived	hydrophilic	high
R	Arginine	Arg	basic	highly hydrophilic	high
S	Serine	Ser	aliphatic hydroxyl	hydrophilic	high
T	Threonine	Thr	aliphatic hydroxyl	hydrophilic	high
U					
V	Valine	Val	aliphatic	hydrophobic	low
W	Tryptophan	Trp	aromatic	highly hydrophobic	low
X					
Y	Tyrosine	Tyr	aromatic	mod. hydrophilic	high
Z	Glu or Gln	Glx			

ACD	Any Acidic
ALP	Any Aliphatic
ALH	Any Aliphatic Hydroxyl
ARO	Any Aromatic
BAS	Any Basic
HY-	Hydrophobic
HY+	Hydrophilic

0360360.0024.0

Fig. 7.1



Position	Tm12.84-2.2	Tm12.84-2.3	Tm12.84-3.4	Tm12.84-3.9	Tm12.84-7.5	Concensus of Tm12.84	Tm13.17	Concensus with Tm 13.17	B1	Concensus with B1	AFP-3	Concensus with AFP-3
1	A	A	A	A	A	A	A	A		A	C	N
2	C	C	C	C	C	C	C	N		N	A	N
3	G	G	G	G	G	G	G	R		R	G	R
4	A	A	A	A	A	A	A	N		N	A	N
5	G	G	G	G	G	G	C	N		N	T	N
6	C	C	C	C	C	C	T	N		N	C	N
7	A	A	A	A	A	A	A	N		N	G	N
8	A	A	A	A	A	A	C	N		N	A	N
9	A	A	A	A	A	A	T	N		N	A	N
10	A	A	A	A	A	A	A	A		A	A	A
11	A	A	A	A	A	A	G	R		R	G	R
12	*	*	*	*	*	*	*	*		*	*	*
13	A	A	A	A	A	A	A	A	A?	A	A	A
14	T	T	T	T	T	T	G	T	T?	T	G	T
15	G	G	G	G	G	G	A	G	G?	G	A	G
16	A	A	A	A	A	A	A	A		A	A	A
17	A	A	A	A	A	A	A	A		A	A	A
18	A	A	A	A	A	A	G	R		R	G	R
19	C	C	C	C	C	C	T	Y		Y	C	Y
20	T	T	T	T	T	T	T	T		T	T	T
21	C	C	C	C	C	C	G	C/G		C/G	C	C/G
22	C	C	C	C	C	C	C	C		C	C	C
23	C	C	C	C	C	C	T	C		C	T	C
24	C	C	C	C	C	C	C	C		C	C	C
25	T	T	T	T	T	T	T	T		T	T	Y
26	T	T	T	T	T	T	N	N		N	C	N
27	G	G	G	G	G	G	T	T		T	T	T
28	G	G	G	G	G	G	G	G		G	G	G
29	C	C	C	C	C	C	T	Y		Y	C	Y
30	T	T	T	T	T	T	T	T		T	T	T
31	T	T	T	T	T	T	T	T	C	T	T	T
32	T	T	T	T	T	T	A	T/A	T	T/A	G	T/A
33	T	T	T	T	T	T	A	R	A	R	T	R
34	G	G	G	G	G	G	T	Y	C	Y	G	Y
35	C	C	C	C	C	C	C	N	T	N	T	N
36	G	G	G	G	G	G	T	Y	T	Y	C	Y
37	T	T	T	T	T	T	T	Y	T	Y	T	Y
38	T	T	T	T	T	T	C	Y	C	Y	C	Y
39	C	C	C	C	C	C	C	C	T	C	T	C
40	G	G	G	G	G	G	G	G/C	C	G/C	C	G/C
41	C	C	C	C	C	C	C	Y	T	Y	C	Y
42	C	C	C	C	C	C	C	C	C	C	T	Y
43	G	G	G	G	G	G	C	R	A	R	G	R
44	C	C	C	C	C	C	C	Y	T	Y	C	Y
45	C	C	C	C	C	C	C	Y	T	Y	T	Y
46	A	A	A	A	A	A	A	N	C	N	T	N
47	T	T	T	T	T	T	T	T	T	T	G	T
48	C	C	C	C	C	C	G	C/G	G	C/G	C	C/G
49	G	G	G	G	G	G	T	N	T	N	T	N
50	T	T	T	T	T	T	T	T	G	T	T	T
51	C	C	C	C	C	C	G	C/G	G	C/G	C	C/G
52	A	A	A	A	A	A	A	R	G	R	G	R
53	T	T	T	T	T	T	C	T	T	T	C	Y
54	C	C	C	C	C	C	C	C	C	C	T	C
55	G	G	G	G	G	G	R	N	C	N	C	N
56	G	G	G	G	G	G	G/C	G/C	C	G/C	G	G/C
57	A	A	A	A	A	A	A	A	A	A	C	R
58	G	G	G	G	G	G	G	G	G	G	G	G
59	C	C	C	C	C	C	Y	Y	A	Y	A	Y
60	T	T	T	T	T	T	T	T	G	T	C	Y
61	C	C	C	C	C	C	C	C	T	C	C	C
62	A	A	A	A	A	A	A	A	A	A	T	A
63	G	G	G	G	G	G	G	G	G	G	A	G
64	G	G	G	G	G	G	G	G	G	G	C	G
65	C	C	C	C	C	C	C	C	C	C	T	C
66	T	T	T	T	T	T	C	Y	C	Y	A	Y
67	C	C	C	C	C	C	T	C	A	C	C	C
68	T	T	T	T	T	T	A	T	T	T	G	T
69	C	C	C	C	C	C	C/G	C/G	A	C/G	C	C/G
70	A	A	A	A	A	A	A	A	C	A	G	A
71	C	C	C	C	C	C	C	C	C	C	A	C
72	C	C	C	C	C	C	G	C	T	C	A	C
73	G	G	G	G	G	G	A	G	C	G	G	G
74	A	A	A	A	A	A	G	A	A	A	A	A
75	C	C	C	C	C	C	A	N	G	N	A	N
76	G	G	G	G	G	G	G	G	G	G	C	R
77	A	A	A	A	A	A	A	N	A	N	A	N
78	A	A	A	A	A	A	C	A	A	A	A	A
79	C	C	C	C	C	C	A	C	A	C	C	C/G
80	A	A	A	A	A	A	G	A	G	A	C	N
81	G	G	G	G	G	G	A	R	A	A	C	N
82	A	A	A	A	A	A	C	A	C	C	T	N
83	T	T	T	T	T	T	A	T	T	T	C	N
84	A	A	A	A	A	A	T	A/T	T	A/T	G	A/T
85	C	C	C	C	C	C	G	C/G	G	C/G	T	C/G
86	A	A	A	A	A	A	A	A	A	A	G	A
87	G	G	G	G	G	G	G	G	G	G	A	G
88	A	A	A	A	A	A	A	A	G	N	G	N
89	A	A	A	A	A	A	A	A	C	A/T	A	A/T
90	A	A	A	A	A	A	A	A	T	A	G	R
91	A	A	A	A	A	A	G	N	A	N	C	N
92	G	G	G	G	G	G	G	G	C	G	T	G
93	G	G	G	G	G	G	A	G	C	N	G	N
94	A	A	A	A	A	A	A	A	C	N	A	N
95	A	A	A	A	A	A	C	A	C	N	A	N
96	C	C	C	C	C	C	A	C	A	C	A	Y
97	A	A	A	A	A	A	A	A	A	A	G	A
98	A	A	A	A	A	A	G	A	A	A	C	A
99	G	G	G	G	G	G	A	G	C	A	A	G
100	A	A	A	A	A	A	T	A	A	A	G	R
101	T	T	T	T	T	T	C	T	A	A	C	A
102	C	C	C	C	C	C	A	C	A	C	A	C
103	A	A	A	A	A	A	G	A	G	A	C	A
104	G	G	G	G	G	G	C	G	C	G	A	G
105	C	C	C	C	C	C	A	C	G	C	G	C
106	A	A	A	A	A	A	A	A	C	A	C	R
107	A	A	A	A	A	A	A	A	A	A	G	N
108	A	A	A	A	A	A	G	A	G	A	C	N
109	G	G	G	G	G	G	A	R	A	R	G	R
110	A	A	A	A	A	A	A	A	A	A	C	N
111	A	A	A	A	A	A	R	A	R	R	T	N
112	T	T	T	T	T	T	T	T	T	T	G	T
113	G	G	G	G	G	G	T	Y	G	Y	C	Y
114	C	C	C	C	C	C	G	C	C	C	A	C
115	C	C	C	C	C	C	T	Y	A	Y	A	Y
116	A	A	A	A	A	A	C	A	A	A	A	A
117	G	G	G	G	G	G	A	R	G	R	G	R

Fig. 7.2

Position	Tm12.84-2.2	Tm12.84-2.3	Tm12.84-3.4	Tm12.84-3.9	Tm12.84-7.5	Concensus of	Tm13.17	Concensus with	B1	Concensus with	AFP-3	Concensus with
118	C	C	C	C	C	C	A	N	A	N	G	N
119	A	A	A	A	A	A	A	A	A	N	C	N
120	G	G	G	G	G	G	T	N	T	N	T	N
121	G	G	G	G	G	G	G	G	G	G	G	G
122	T	G	T	A	T	T/A	A	T/A	A	T/A	A	A/T
123	T	G	T	G	T	G	A	A	A	A	A	R
124	T	C	C	T	C	T/A	A	T/A	G	T/A	T	T/A
125	C	C	C	C	C	C	G	C/G	G	C/G	C	C/G
126	C	C	C	C	C	C	T	Y	T	Y	T	Y
127	G	G	G	G	G	G	G	G	G	G	G	G
128	G	G	G	G	G	G	G	G	G	G	G	G
129	A	A	A	A	A	A	A	A	A	A	A	A
130	T	G	T	G	T	G	T	G	T	G	T	G
131	T	G	T	G	T	G	T	G	T	G	T	R
132	T	C	C	T	C	T	G	T	G	T	A	T
133	T	C	C	T	C	T	C	C	C	C	T	C
134	C	C	C	C	C	C	C	C	C	C	C	C
135	C	C	C	C	C	C	G	C/G	C	C/G	T	N
136	C	C	C	C	C	C	C	C	C	C/G	G	C/G
137	A	A	A	A	A	A	A	A	A	A	A	A
138	A	A	A	A	A	A	A	A	A	A	A	A
139	G	G	G	G	G	G	G	G	G	G	G	G
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141	G	G	G	G	G	G	G	G	C	G/C	G	G/C
142	A	A	A	A	A	A	A	A	G	R	T	N
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144	G	G	G	G	G	G	C	G/C	C	G/C	C	G/C
145	A	A	A	A	A	A	A	A	A	A	C	N
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147	C	G	C	G	C	C	A	N	A	N	C	N
148	G	A	G	A	G	G	A	R	A	R	A	R
149	A	C	A	C	A	A	C	N	A	N	A	N
150	C	A	C	A	C	C	C	C	G	C/G	C	C/G
151	A	A	A	A	A	A	A	A	A	A	A	A
152	A	A	A	A	A	A	A	A	A	A	A	R
153	A	G	A	G	A	A	G	A	G	A	G	R
154	G	T	G	T	G	G	T	G	C	G	T	G
155	T	C	T	C	T	T	C	Y	T	Y	T	Y
156	C	C	C	C	C	C	C	Y	C	Y	C	Y
157	C	G	C	G	C	C	G	C	T	C	T	C
158	C	G	C	G	C	C	G	C	G	G	C	G
159	C	C	C	C	C	C	C	C	C	C	C	C
160	A	A	A	A	A	A	A	A	A	A	A	A
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162	A	A	A	A	A	A	A	N	A	N	A	N
163	G	G	G	G	G	G	G	G	G	G	C	G/C
164	G	G	G	G	G	G	G	G	G	G	G	G
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166	T	G	T	G	T	G	T	G	G	G	T	G
167	T	C	T	C	T	T	C	T/A	A	T/A	A	T/A
168	T	C	T	C	T	T	C	C	T	C	T	N
169	T	T	T	T	T	T	T	T	G	T	G	N
170	T	T	T	T	T	T	T	N	G	N	A	N
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172	G	T	G	G	G	G	G	G	G	G	G	G
173	T	G	T	G	T	G	A	T/A	A	T/A	T	T/A
174	C	G	C	G	C	C	G	C/G	G	C/G	G	C/G
175	G	A	G	A	G	G	A	G	G	G	G	G
176	A	C	A	C	A	A	Y	A	C	A	G	A
177	T	G	T	G	T	T	G	Y	G	Y	G	Y
178	T	G	T	G	T	T	G	G	G	G	G	G
179	A	T	A	T	A	A	T	A	A	A	G	A
180	T	C	T	C	T	T	C	T	A	T	A	A
181	C	C	C	C	C	C	C	C	C	C	C	Y
182	C	C	C	C	C	C	C	C	C	C	C	C
183	C	C	C	C	C	C	C	Y	C	Y	C	Y
184	A	A	A	A	A	A	A	A	A	A	A	A
185	A	A	A	A	A	A	A	A	A	A	A	A
186	A	A	A	A	A	A	A	A	A	A	A	A
187	A	A	A	A	A	A	A	N	A	N	A	N
188	T	G	T	G	T	T	A	T	C	T	C	T
189	G	A	G	A	G	G	A	G	T	G	T	G
190	A	A	A	A	A	A	A	A	G	A	G	A
191	A	A	A	A	A	A	A	A	A	A	A	A
192	G	A	G	A	G	G	A	R	A	R	A	R
193	A	A	A	A	A	A	C	N	T	N	G	N
194	A	A	A	A	A	A	G	R	G	N	A	N
195	G	C	G	C	G	G	C	G/C	C	G/C	G	G/C
196	C	A	C	A	C	C	C	C	A	C	C	C
197	A	C	A	C	A	A	C	A	A	A	C	A
198	C	G	C	G	C	C	G	N	A	N	A	N
199	G	T	G	T	G	G	T	G	C	G/C	T	G/C
200	T	C	T	C	T	T	C	T	T	T	C	Y
201	C	C	C	T	C	C	T	Y	T	Y	T	Y
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203	T	C	T	C	T	T	C	T	T	T	C	T
204	C	T	C	T	C	C	T	Y	T	Y	T	Y
205	T	G	T	G	T	T	G	T	T	T	G	T
206	G	C	G	C	G	G	C	G	T	G	T	G
207	C	T	C	T	C	C	T	C	G	C	G	C
208	T	T	T	T	T	T	T	N	C	N	C	N
209	T	C	T	C	T	T	C	T	T	T	A	T
210	C	T	C	T	C	C	T	C/G	G	N	T	N
211	T	C	T	C	T	T	C	N	G	N	T	N
212	C	G	C	G	C	C	G	C	T	C	Y	Y
213	G	A	G	A	G	G	A	G/C	C	G/C	T	G/C
214	A	G	A	G	A	A	G	A	A	A	G	A
215	A	A	A	A	A	A	A	R	G	R	A	R
216	G	A	G	A	G	G	A	G	A	G	A	G
217	A	A	A	A	A	A	A	A	A	A	A	R
218	A	A	A	A	A	A	A	R	G	R	G	N
219	A	A	A	A	A	A	A	A	C	N	A	N
220	A	C	A	C	A	A	C	N	A	N	A	N
221	C	T	C	T	C	C	T	C	C	C	T	Y
222	T	G	T	G	T	T	G	Y	C	Y	C	Y
223	G	A	G	A	G	G	A	G	G	G	C	G
224	G	G	G	G	G	G	G	G	G	G	G	R
225	A	G	A	G	A	A	G	A/T	T	A/T	G	A/T
226	G	T	G	T	G	G	T	G/C	C	N	A	N
227	T	G	T	G	T	T	G	T	T	T	T	T
228	T	G	T	G	T	T	G	G	A	R	C	N
229	G	C	G	C	G	G	C	G	T	G	A	R
230	C	A	C	A	C	C	A	C	A	Y	T	Y
231	A	C	A	C	A	A	C	N	G	N	C	N
232	A	C	A	C	A	A	C	A	T	R	G	R
233	C	C	C	C	C	C	C	C	C	C	A	N
234	C	C	C	C	C	C	C	C/G	G	C/G	C	C/G
235	G	G	G	G	G	G	G	G	G	G	G	G

Fig. 7.2 cont.

Position	Tm12.84-2.2	Tm12.84-2.3	Tm12.84-3.4	Tm12.84-3.9	Tm12.84-7.5	Concensus of	Tm13.17	Concensus with	B1	Concensus with	AFP-3	Concensus with
236	A	A	A	A	A	A	A	A	A	A	C	N
237	A	A	A	A	A	A	A	A	A	A	C	N
238	G	G	G	G	G	G	T	N	T	N	A	N
239	C	C	C	C	C	C	C	C	G	C	G	C/G
240	C	C	C	C	C	C	G	C/G	G	C/G	T	N
241	G	G	G	G	G	G	G	G	G	G	G	G
242	G	G	G	G	G	G	A	G	A	G	G	A/T
243	A	A	A	A	A	A	G	A	G	A	T	G
244	G	G	G	G	G	G	A	G	A	A	G	A
245	A	A	A	A	A	A	G	A	A	N	A	A
246	C	C	C	C	C	C	G	C/G	A	R	A	N
247	A	A	A	A	A	A	G	R	A	Y	T	N
248	C	C	C	C	C	C	T	C/G	T	N	T	N
249	C	C	C	C	C	C	G	R	G	R	C	N
250	A	A	A	A	A	A	T	A/T	A	R	A	A/T
251	A	A	A	A	A	A	G	N	G	N	A	N
252	T	T	T	T	T	T	G	G	G	G	C	G/C
253	G	G	G	G	G	G	T	G	C	Y	T	Y
254	T	T	T	T	T	T	C	G/C	C	G/C	G	G/C
255	G	G	G	G	G	G	G	A	G	A	G	A
256	G	G	G	G	G	G	A	G/C	A	G/C	A	G/C
257	A	A	A	A	A	A	G	R	C	R	C	N
258	G	G	G	G	G	G	T	R	A	Y	A	N
259	G	G	G	G	G	G	T	R	C	R	T	N
260	T	T	T	T	T	T	T	Y	G	Y	T	N
261	A	A	A	A	A	A	T	Y	T	Y	A	T
262	C	C	C	C	C	C	C	C/G	C	C/G	T	N
263	T	T	T	T	T	T	A	A	A	A	A	R
264	C	C	C	C	C	C	A	R	A	R	A	R
265	A	A	A	A	A	A	G	R	G	N	G	N
266	A	A	A	A	A	A	G	G	A	G	A	G/C
267	A	A	A	A	A	A	G	A	G	A	A	R
268	G	G	G	G	G	G	A	A	A	A	A	R
269	C	C	C	C	C	C	A	A	A	A	T	T
270	C	C	C	C	C	C	A	A	G	N	T	G/C
271	A	A	A	A	A	A	G	A	G	A	G	A
272	A	A	A	A	A	A	G	A	A	G	A	A
273	G	G	G	G	G	G	C	G	A	G	A	R
274	C	C	C	C	C	C	T	C/G	T	G	T	N
275	T	T	T	T	T	T	G	T	T	G	C	G/C
276	G	G	G	G	G	G	A	G	A	N	A	A
277	A	A	A	A	A	A	A	A	C	G	A	N
278	A	A	A	A	A	A	G	R	G	N	G	N
279	G	G	G	G	G	G	C	G	N	N	G	N
280	C	C	C	C	C	C	A	A	G	N	N	R
281	A	A	A	A	A	A	T	A	A	G	N	N
282	T	T	T	T	T	T	G	T	G	T	N	N
283	G	G	G	G	G	G	T	G	T	G	N	N
284	T	T	T	T	T	T	G	T	G	A	A	A
285	G	G	G	G	G	G	T	G/C	C	A	C	T
286	G	G	G	G	G	G	C	R	A	A	T	C
287	C	C	C	C	C	C	C	C	A	A	C	T
288	C	C	C	C	C	C	C	Y	A	A	T	C
289	A	A	A	A	A	A	A	R	C	G	G	T
290	G	G	G	G	G	G	G	R	G	A	A	G
291	C	C	C	C	C	C	C	C	A	T	G	A
292							C	A	T	G	A	G
293							A	A	A	T	A	G
294							C	C	T	G	T	A
295	G	G	G	G	G	G	G	G	A	T	G	A
296	A	A	A	A	A	A	G	A	T	G	T	A
297	C	C	C	C	C	C	A	C	G	A	G	A
298	G	G	G	G	G	G	A	G	A	G	A	G
299	A	A	A	A	A	A	A	A	G	A	A	A
300	A	A	A	A	A	A	G	A	A	G	A	R
301	G	G	G	G	G	G	A	G	A	A	A	R
302	A	A	A	A	A	A	G	A	A	A	A	R
303	G	G	G	G	G	G	A	R	A	R	A	R
304	G	G	G	G	G	G	T	G	A	R	A	R
305	T	T	T	T	T	T	G	T	A	R	G	R
306	G	G	G	G	G	G	T	G	C	Y	T	Y
307	G	G	G	G	G	G	A	G	G	N	C	N
308	A	A	A	A	A	A	C	A	A	R	G	R
309	C	C	C	C	C	C	A	C	G	R	A	R
310	A	A	A	A	A	A	A	A	A	N	T	N
311	A	A	A	A	A	A	G	A	A	A	G	R
312	G	G	G	G	G	G	A	G	A	R	A	R
313	A	A	A	A	A	A	G	A	T	R	C	N
314	T	T	T	T	T	T	T	A	T	A/T	T	A/T
315	C	C	C	C	C	C	T	C	T	T	T	T
316	G	G	G	G	G	G	C	R	G	C/G	G	C/G
317	T	T	T	T	T	T	G	T	T	N	T	N
318	G	G	G	G	G	G	T	G/C	C	Y	G	Y
319	C	C	C	C	C	C	G	C	G	G/C	T	N
320	A	A	A	A	A	A	C	A	A	N	G	N
321	G	G	G	G	G	G	A	G	A	R	T	N
322	A	A	A	A	A	A	G	A	A	N	G	N
323	A	A	A	A	A	A	A	A	A	A	C	A
324	G	G	G	G	G	G	A	G	A	R	A	R
325	T	T	T	T	T	T	G	T	G	N	A	N
326	G	G	G	G	G	G	T	G	T	N	A	N
327	C	C	C	C	C	C	G	C	G	G/C	T	G/C
328	G	G	G	G	G	G	T	G	C	N	G	N
329	T	T	T	T	T	T	G	T	G	G/C	C	G/C
330	G	G	G	G	G	G	C	G	C	N	C	N
331	G	G	G	G	G	G	G	T	G	T	G	G/C
332	T	T	T	T	T	T	T	C	T	N	T	N
333	C	C	C	C	C	C	A	A	G	N	C	N
334	A	A	A	A	A	A	A	A	A	N	A	N
335	A	A	A	A	A	A	G	A	G	N	A	N
336	G	G	G	G	G	G	A	G	A	G	A	G
337	A	A	A	A	A	A	G	A	A	A	A	A
338	A	A	A	A	A	A	G	A	R	R	A	R
339	G	G	G	G	G	G	A	G	A	N	G	N
340	G	G	G	G	G	G	G	G	C	R	A	R
341	C	C	C	C	C	C	G	C	C	N	G	N
342	C	C	C	C	C	C	C	C	C	N	A	N
343	C	C	C	C	C	C	A	C	A	N	C	N
344	A	A	A	A	A	A	C	A	C	Y	A	Y
345	C	C	C	C	C	C	A	C	T	N	C	N
346	C	C	C	C	C	C	C	C	C	C/G	T	C/G
347	C	C	C	C	C	C	A	C	G	N	C	N
348	A	A	A	A	A	A	G	A	G	N	T	N
349	G	G	G	G	G	G	A	G	A	R	C	N
350	A	A	A	A	A	A	G	A	A	A	A	A
351	G	G	G	G	G	G	G	A	G	R	G	R
352	G	G	G	G	G	G	A	R	A	R	C	N
353	A	A	A	A	A	A	A	A	T	A/T	A	A/T

Fig. 7.2 cont.

Position	Tm12.84-2.2	Tm12.84-2.3	Tm12.84-3.4	Tm12.84-3.9	Tm12.84-7.5	Concensus of	Tm13.17	Concensus with	B1	Concensus with	AFP-3	Concensus with
354	A	A	A	A	A	A	G	R	A	R	C	N
355	A	A	A	A	A	A	A	A	C	N	T	N
356	C	C	G	C	C	C	C	C	G	C/G	C	C/G
357	G	G	G	G	G	G	G	G	C	G/C	T	G/C
358	G	G	G	G	G	G	G	G	A	G/C	C	N
359	C	C	C	C	C	C	T	Y	T	N	A	N
360	T	T	T	T	T	T	G	N	T	N	G	N
361	T	T	T	T	T	T	T	T	G	T	C	N
362	A	A	A	A	A	A	T	A/T	A	A/T	C	N
363	T	T	T	T	T	T	C	Y	A	N	G	N
364	G	G	G	G	G	G	A	R	G	R	C	R
365	A	A	A	A	A	A	A	A	C	A	G	A
366	C	C	C	C	C	C	T	Y	A	N	A	A/T
367	A	A	A	A	A	A	A	A	G	A/T	C	Y
368	C	C	C	C	C	C	C	C	T	Y	T	N
369	C	C	C	C	C	C	T	Y	A	N	C	Y
370	T	T	T	T	T	T	T	T	C	Y	T	Y
371	T	T	T	T	T	T	C	Y	C	N	T	N
372	C	C	C	C	C	C	A	A	A	A	T	A
373	A	A	A	A	A	A	A	A	A	N	A	A
374	A	A	A	A	A	A	A	A	A	N	A	N
375	G	G	G	G	G	G	A	R	A	N	A	N
376	T	T	T	T	T	T	T	N	A	N	A	N
377	G	G	G	G	G	G	N	N	A	N	A	N
378	T	T	T	T	T	T	N	N	A	N	A	N
379	A	A	A	A	A	A	T	R	A	N	A	N
380	T	T	T	T	T	T	A	T	T	Y	G	T/A
381	T	T	T	T	T	T	T	Y	G	T/A	T	Y
382	T	T	T	T	T	T	G	T/A	A	T/A	T	N
383	A	A	A	A	A	A	T	A/T	G	N	C	N
384	C	C	C	C	C	C	G	C/G	A	N	A	N
385	G	G	G	G	G	G	A	R	G	R	C	R
386	A	A	A	A	A	A	A	A	A	R	G	R
387	C	C	C	C	C	C	A	N	C	N	A	N
388	A	A	A	A	A	A	A	A	A	N	T	A
389	G	G	G	G	G	G	G	R	A	N	C	N
390	C	C	C	C	C	C	A	Y	G	N	A	N
391	A	A	A	A	A	A	A	A	A	N	C	A
392	A	A	A	A	A	A	A	A	G	R	A	R
393	A	A	A	A	A	A	A	R	A	N	C	N
394	C	C	C	C	C	C	G	C	G	C	A	Y
395	C	C	C	C	C	C	C	C	C	C	G	C
396	T	T	T	T	T	T	A	T/A	A	T/A	G	N
397												
398	G	G	G	G	G	G	A	R	A	R		
399	A	A	A	A	A	A	A	A	T	A/T		
400	T	T	T	T	T	T	G	A	T	N		
401	T	T	T	T	T	T	T	T	T	T		
402	T	T	T	T	T	T	T	T	C	Y		
403	C	C	C	C	C	C	T	Y	T	T		
404	T	T	T	T	T	T	C	C	T	Y		
405	C	C	C	C	C	C	A	A	T	N		
406	T	T	T	T	T	T	C	C	T	C		
407	C	C	C	C	C	C	C	C	T	N		
408	C	C	C	C	C	C	A	A	G	C		
409	T	T	T	T	T	T	G	G	C	N		
410	A	A	A	A	A	A	T	T	A	N		
411	T	T	T	T	T	T	T	T	G	R		
412	T	T	T	T	T	T	T	T	A	Y		
413	G	G	G	G	G	G	G	G	C	T		
414	A	A	A	A	A	A	A	A	T	R		
415	T	T	T	T	T	T	T	T	T	A/T		
416	-	-	-	-	-	-	-	-	-	-	-	-
417	T	T	T	T	T	T	T	T	T	T	T	T
418	A	A	A	A	A	A	G	R	A	R	A	R
419	A	A	A	A	A	A	A	A	G	A/T	A	A/T
420	T	T	T	T	T	T	A	T/A	C	N	T	N
421	T	T	T	T	T	T	C	Y	T	Y	G	N
422	G	G	G	G	G	G	C	G/C	A	G/C	G	N
423	T	T	T	T	T	T	A	T/A	G	T/A	G	N
424	T	T	T	T	T	T	C	Y	A	N	G	N
425	T	T	T	T	T	T	T	T	G	N	T	N
426	T	T	T	T	T	T	G	T/A	C	N	C	N
427	G	G	G	G	G	G	A	G/C	A	G/C	G	N
428	T	T	T	T	T	T	G	N	G	N	C	N
429	A	A	A	A	A	A	A	A	A	A/T	G	N
430	T	T	T	T	T	T	T	Y	G	Y	A	Y
431	T	T	T	T	T	T	T	T	A	T/A	G	N
432	G	G	G	G	G	G	R	G	G	G	A	T/A
433	A	A	A	A	A	A	C	N	G	N	G	R
434	C	C	C	C	C	C	T	T	A	N	A	N
435	T	T	T	T	T	T	G	G	G	N	G	N
436	G	G	G	G	G	G	A	A	A	R	A	A/T
437	A	A	A	A	A	A	A	A	A	A/T	C	A/T
438	A	A	A	A	A	A	T	R	G	R	T	R
439	T	T	T	T	T	T	T	N	C	N	T	N
440	T	T	T	T	T	T	T	T	T	T	A	T/A
441	T	T	T	T	T	T	T	T	T	Y	C	T/A
442	T	T	T	T	T	T	G	R	A	R	T	Y
443	T	T	T	T	T	T	A	A	T	A	T	R
444	G	G	G	G	G	G	A	A	A	N	A	A
445	A	A	A	A	A	A	C	N	A	N	A	N
446	C	C	C	C	C	C	C	C	A	N	T	N
447											G	R
448											T	T
449											G	A
450											G	A
451											T	T
452											C	A
453											G	A
454											C	A
455											G	A
456											A	A
457											C	A
458											T	A
459											T	A
460											A	A
461											A	A
462											T	A
463											A	A
464											T	A
465											A	A
466											A	A
467											A	A
468											A	A
469											A	A
470											A	A
471											C	G/C

Fig. 7.2 cont.

Position	Tm12.84-2.2	Tm12.84-2.3	Tm12.84-3.4	Tm12.84-3.9	Tm12.84-7.5	Concensus of	Tm13.17	Concensus with	B1	Concensus with	AFP-3	Concensus with
472	A	A	A	A	A	A	A	A		A	A	A
473	A	A	A	A	A	A	A	A		A	A	A
474	T	T	T	T	T	T	T	T		T	T	T
475	A	A	A	A	A	A	A	A		A	A	A
476	A	A	A	A	A	A	A	A		A	A	A
477	A	A	A	A	A	A	A	A		A	A	A
478	G	G	G	G	G	G	G	G		G	T	N
479	G	G	G	G	G	G	T	N		N	T	N
480	T	T	T	T	T	T	G	N		N	T	N
481	A	A	A	A	A	A	T	N		N	A	N
482	A	C	C	C	C	C	T	Y		Y	A	N
483	T	T	T	T	T	T	T	T		T	A	A/T
484	A	A	A	A	A	A	C	N		N	G	N
485	T	T	T	T	T	T	T	T		T	A	A/T
486	C	C	C	C	C	C	G	N		N	A	N
487	G	G	G	G	G	G	A	R		R	A	R
488	T	T	T	T	T	T	T	T		T	A	T/A
489	T	T	T	T	T	T	G	N		N	A	N
490	A	A	A	A	A	A	T	N		N	A	N
491	T	T	T	T	T	T	A	N		N	A	R
492	G	G	G	G	G	G	A	R		R	A	N
493	T	A	T	A	T	N	A	N		N	A	N
494	A	A	A	A	A	A	A	A		A	A	A
495	A	A	A	A	A	A	A	A		A	A	A
496	A	A	A	A	A	A	A	A		A	A	A
497	A	A	A	A	A	A	A	A		A	A	A
498	A	A	A	A	A	A	A	A		A	A	A
499	A	A	A	A	A	A	A	A		A	A	A
500	A	A	A	A	A	A	A	A		A	A	A
501	A	A	A	A	A	A	A	A		A	A	A
502	A	A	A	A	A	A	A	A		A	A	A
503	A	A	A	A	A	A	A	A		A		A
504	A	A	A	A	A	A	A	A		A		A
505	A	A	A	A	A	A	A	A		A		A
506	A	A	A	A	A	A	A	A		A		A
507	A	A	A	A	A	A	A	A		A		A
508	A	A	A	A	A	A	A	A		A		A
509	A	A	A	A	A	A	A	A		A		A
510	A	A	A	A	A	A	A	A		A		A
511	A	A	A	A	A	A	A	A		A		A
512												

Fig. 7.2 cont.

Position	Tm 12.84-2.2	Tm 12.84-2.3	Tm 12.84-3.4	Tm 12.84-3.9	Tm 12.84-7.5	Concensus Tm 12.84	Tm 13.17	Concensus to Tm13.17	Tm P-B1	Tm P-B2	Concensus to B1/B2	Tm AFP-3	Concensus to AFP-3	GENERAL CONCENSUS	SUBSTITUTIONS - most to least common			
1	M	M	M	M	M	M	M	M			M, OR GAP	M	M, OR GAP	M				
2	K	K	K	K	K	K	K	K			K, OR GAP	K	K, OR GAP	K				
3	L	L	L	L	L	L	L	L			L, OR GAP	L	L, OR GAP	L				
4	L	L	L	L	L	L	L	L			L, OR GAP	L	L, OR GAP	L				
5	L	L	L	L	L	L	C	HY-, ALP/SUL			HY-, ALP/SUL, OR G	L	-, ALP/SUL, OR GAP	L	C			
6	C	C	C	C	C	C	C	C			C OR GAP	C	C OR GAP	C				
7	F	F	F	F	F	F	L	HY-, ARO/ALP	L	L	HY-, ARO/ALP	L	HY-, ARO/ALP	F	L			
8	A	A	A	A	A	A	I	ALP	T	T	ALP/ALH	V	ALP/ALH	A	I	T	V	
9	F	F	F	F	F	F	S	ARO/ALH	S	S	ARO/ALH	L	ARO/ALH/ALP	F	S	L		
10	A	A	A	A	A	A	L	ALP	L	L	ALP	V	ALP	A	L	V		
11	A	A	A	A	A	A	I	ALP	I	I	ALP	A	ALP	A	I			
12	I	I	I	I	I	I	L	ALP	L	L	ALP	L	ALP	I	L			
13	V	V	V	V	V	V	L	ALP	L	L	ALP	V	ALP	V	L			
14	I	I	I	I	I	I	V	ALP	V	V	ALP	A	ALP	I	V	A		
15	G	G	G	G	G	G	T	ALP/ALH	A	A	ALP/ALH	A	ALP/ALH	G	T	A		
16	A	A	A	A	A	A	V	ALP	V	V	ALP	T	ALP/ALH	A	V	T		
17	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Y	ACD/ARO	Q	Y			
18	A	A	A	A	A	A	A	A	A	A	A	A	A	A				
19	L	L	L	L	L	L	L	L	I	L	ALP		ALP OR GAP	L	I			
20	T	T	T	T	T	T	T	T	T	T	T		T OR GAP	T				
21	D	D	D	D	D	D	E	ACD	E	E	ACD	E	ACD	D	E			
22	E	E	E	E	E	E	A	ACD/ALP	E	E	ACD/ALP	T	ACD/ALP/ALH	E	A	T		
23	Q	Q	Q	Q	Q	Q	Q	Q	D	D	ACD	P	ACD/ALP	Q	D	P		
24	I	I	I	I	I	I	I	I	L	L	ALP	R	ALP/BAS	I	L	R		
25	Q	Q	Q	Q	Q	Q	E	GLX	E	Q	GLX	E	GLX	Q	E			
26	K	K	K	K	K	K	K	K	L	L	BAS/ALP	K	BAS/ALP	K	L			
27	R	R	R	R	R	R	L	BAS/ALP	L	L	BAS/ALP	L	BAS/ALP	R	L			
28	N	N	N	N	N	N	N	N	R	R	HY+, BAS/ACD	K	HY+, BAS/ACD	N	R	K		
29	K	K	K	K	K	K	K	K	Q	Q	HY+, BAS/ACD	Q	HY+, BAS/ACD	K	Q			
30	I	I	I	I	I	I	I	I	T	T	ALP/ALH	H	ALP/ALH/BAS	I	T	H		
31	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
32	K	K	K	K	K	K	K	K	A	A	BAS/ALP	D	BAS/ALP/ACD	K	A	D		
33	E	E	E	E	E	E	K	HY+, ACD/BAS	E	E	HY+, ACD/BAS	A	ACD/BAS/ALP	E	K	A		
34	C	C	C	C	C	C	C	C	C	C	C	C	C	C				
35	Q	Q	Q	Q	Q	Q	Q	Q	K	K	HY+, ACD/BAS	K	HY+, ACD/BAS	Q	K			
36	Q	Q	Q	Q	Q	Q	N	ACD	T	T	HY+, ACD/ALH	A	ACD/ALH/ALP	Q	N	T	A	
37	V	V	V	E	V	ALP/ACD	E	ACD/ALP	E	E	ACD/ALP	E	ACD/ALP	E	V	(3.9, Tm13.17, B1, B2, AFP-3)		
38	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
39	G	G	G	G	G	G	G	G	G	G	G	G	G	G				
40	V	V	V	V	V	V	V	V	V	A	ALP	V	ALP	V	A			
41	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
42	Q	Q	Q	Q	Q	Q	Q	Q	E	E	GLX	E	GLX	Q	E			
43	E	E	E	E	E	E	E	E	D	A	ACD/ALP	E	ACD/ALP	E	D	A		
44	T	T	T	T	T	T	I	ALP	V	V	ALP	S	ALP/ALH	T	I	V	S	
45	I	I	I	I	I	I	I	I	I	I	I	L	ALP	I	L			
46	D	D	D	D	D	D	T	HY+, ACD/ALH	K	K	Y+, ACD/ALH/BA	N	Y+, ACD/ALH/BAS	D	T	K	N	
47	K	K	K	K	K	K	K	K	R	K	BAS	K	BAS	K	R			
48	V	V	V	V	V	V	A	ALP	A	A	ALP	V	ALP	V	A			
49	R	R	R	R	R	R	R	R	R	R	R	R	R	R				
50	T	T	T	T	T	T	N	HY+, ALH/ACD	K	K	Y+, ALH/ACD/BA	N	Y+, ALH/ACD/BAS	T	N	K		
51	G	G	G	G	G	G	G	G	G	G	G	R	ALP/BAS	G	R			
52	V	V	V	V	V	V	D	ALP/ACD	D	D	ALP/ACD	E	ALP/ACD	V	D	E		
53	L	L	L	L	L	L	W	HY-, ALP/ARO	L	L	HY-, ALP/ARO	E	ALP/ARO/ACD	L	W	E		
54	V	V	V	V	V	V	E	ALP/ACD	E	E	ALP/ACD	V	ALP/ACD	V	E			
55	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
56	D	D	D	D	D	D	D	D	D	D	D	D	D	D				
57	P	P	P	P	P	P	P	P	P	P	P	P	P	P				
58	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
59	M	M	M	M	M	M	L	HY-, SUL/ALP	L	L	HY-, SUL/ALP	L	HY-, SUL/ALP	M	L			
60	K	K	K	K	K	K	K	K	K	K	K	K	K	K				
61	K	K	K	K	K	K	R	BAS	M	M	BAS/SUL	E	BAS/SUL/ACD	K	R	M	E	
62	H	H	H	H	H	H	Q	HY+, BAS/ACD	Q	Q	HY+, BAS/ACD	H	HY+, BAS/ACD	H	Q			
63	V	V	V	V	V	V	V	V	L	L	ALP	A	ALP	V	L	A		
64	L	L	L	L	L	L	F	HY-, ALP/ARO	L	L	HY-, ALP/ARO	F	HY-, ALP/ARO	L	F			
65	C	C	C	C	C	C	C	C	C	C	C	C	C	C				
66	F	F	F	F	F	F	V	HY-, ARO/ALP	I	I	HY-, ARO/ALP	I	HY-, ARO/ALP	F	V	I		
67	S	S	S	S	S	S	A	ALH/ALP	F	F	ALH/ALP/ARO	L	ALH/ALP/ARO	S	A	F	L	
68	K	K	K	K	K	K	R	BAS	K	K	BAS	K	BAS	K	R			
69	K	K	K	R	K	BAS	N	HY+, BAS/ACD	A	A	BAS/ACD/ALP	R	BAS/ACD/ALP	K	R	A	N	(2.2, 2.3, 3.4, 7.5)
70	T	T	T	T	T	T	A	ALP	L	L	ALP	A	ALP	T	A	L		
71	G	G	G	G	G	G	G	G	I	E	ALP/ACD	G	ALP/ACD	G	I	E		
72	V	V	V	V	V	V	L	ALP	E	I	ALP/ACD	F	ALP/ACD/ARO	V	L	I	E	F
73	A	A	A	A	A	A	A	A	V	V	ALP	I	ALP	A	V	I		
74	T	T	T	T	T	T	T	T	A	A	ALH/ALP	D	ALH/ALP/ACD	T	A	D		
75	E	E	E	E	E	E	E	E	E	E	E	A	ACD/ALP	E	A			
76	A	A	A	A	A	A	S	ALP/ALH	S	S	ALP/ALH	S	ALP/ALH	S	A			
77	G	G	G	G	G	G	G	G	G	G	G	G	G	G				
78	D	D	D	D	D	D	E	ACD	E	E	ACD	E	ACD	E	D			

Fig. 7.3

79	T	T	T	T	T	T	V	ALP	I	I	ALP	F	ALP/ARO	T	I	V	F
80	N	N	N	N	N	N	V	ACD/ALP	E	E	ACD/ALP	Q	ACD/ALP	N	E	V	Q
81	V	V	V	V	V	V	V	V	A	A	ALP	L	ALP	V	A	L	
82	E	E	E	E	E	E	D	ACD	D	D	ACD	D	ACD	D	E		
83	V	V	V	V	V	V	V	V	T	T	ALP/ALH	H	ALP/ALH/BAS	V	T	H	
84	L	L	L	L	L	L	L	L	F	F	ALP/ARO	1	ALP/ARO	L	F	I	
85	K	K	K	K	K	K	R	BAS	K	K	BAS	K	BAS	K	R		
86	A	A	A	A	A	A	E	ALP/ACD	E	E	ALP/ACD	T	ALP/ACD/ALH	A	E	T	
87	K	K	K	K	K	K	K	K	K	K	K	K	K	K			
88	L	L	L	L	L	L	V	ALP	L	L	ALP	F	ALP/ARO	L	V	F	
89	K	K	K	K	K	K	R	BAS	T	T	BAS/ALH	K	BAS/ALH	K	T	R	
90	H	H	H	H	H	H	K	BAS	R	R	BAS	E	BAS/ACD	H	R	K	E
91	V	V	V	V	V	V	V	V	V	V	V	N	ALP/ACD	V	N		
92	A	A	A	A	A	A	T	ALP/ALH	T	T	ALP/ALH	S	ALP/ALH	A	T	S	
93	S	S	S	S	S	S	D	HY+,ALH/ACD	N	N	HY+,ALH/ACD	E	HY+,ALH/ACD	S	N	D	E
94							N	N OR GAP	D	D	ACD OR GAP	H	ACD OR GAP	D	N	H	
95	D	D	D	D	D	D	D	D	D	D	D	P	ACD/ALP	D	P		
96	E	E	E	E	E	E	E	E	E	E	E	E	E	E			
97	E	E	E	E	E	E	E	E	E	E	E	K	HY+ ACD/BAS	E	K		
98	V	V	V	V	V	V	T	ALP/ALH	S	S	ALP/ALH	V	ALP/ALH	V	S	T	
99	D	D	D	D	D	D	E	ACD	E	E	ACD	D	ACD	D	E		
100	K	K	K	K	K	K	K	K	K	K	K	D	HY+, BAS/ACD	K	D		
101	I	I	I	I	I	I	I	I	I	I	I	L	ALP	I	L		
102	V	V	V	V	V	V	I	ALP	V	V	ALP	V	ALP	V	I		
103	Q	Q	Q	Q	Q	Q	N	ACD	E	E	ACD	A	ACD/ALP	Q	E	N	A
104	K	K	K	K	K	K	K	K	K	K	K	K	K	K			
105	C	C	C	C	C	C	C	C	C	C	C	C	C	C			
106	V	V	V	V	V	V	A	ALP	T	T	ALP/ALH	A	ALP/ALH	V	A	T	
107	V	V	V	V	V	V	V	V	V	V	V	V	V	V			
108	K	K	K	K	K	K	K	K	T	T	HY+, ACD/ALH	K	HY+, ACD/ALH	K	T		
109	K	K	K	K	K	K	R	BAS	E	E	HY+, BAS/ACD	K	HY+, BAS/ACD	K	E	R	
110	A	A	A	A	A	A	D	ALP/ACD	D	D	ALP/ACD	D	ALP/ACD	D	A		
111	T	T	T	T	T	T	T	T	T	T	T	T	T	T			
112	P	P	P	P	P	P	V	ALP	P	P	ALP	P	ALP	P	V		
113	E	E	E	E	E	E	E	E	E	E	E	Q	ACD	E	Q		
114	E	E	E	E	E	E	E	E	D	D	ACD	H	HY+, ACD/BAS	E	D	H	
115	T	T	T	T	T	T	T	T	T	T	T	S	ALH	T	S		
116	A	A	A	A	A	A	V	ALP	A	A	ALP	S	ALP/ALH	A	V	S	
117	Y	Y	Y	Y	Y	Y	F	ARO	F	F	ARO	A	ARO/ALP	Y	F	A	
118	D	D	D	D	D	D	N	ASX	E	E	ACD	D	ACD	D	E	N	
119	T	T	T	T	T	T	T	T	V	V	ALH/ALP	F	ALH/ALP/ARO	T	V	F	
120	F	F	F	F	F	F	F	F	T	T	ARO/ALH	F	ARO/ALH/ARO	F	T		
121	K	K	K	K	K	K	K	K	K	K	K	K	K	K			
122	C	C	V	C	C	HY-, SUL/ALP	C	HY-, SUL/ALP	C	C	HY-, SUL/ALP	C	HY-, SUL/ALP	C	V		(3.4 is the only variant in the family)
123	I	I	I	I	I	I	V	ALP	V	V	ALP	V	ALP	V	I		
124	Y	Y	Y	Y	Y	Y	M	ARO/SUL	L	L	ARO/SUL/ALP	H	RO/SUL/ALP/BAS	Y	L	M	H
125	D	D	D	D	D	D	K	HY+, ACD/BAS	K	K	HY+, ACD/BAS	D	HY+, ACD/BAS	D	K		
126	S	S	S	S	S	S	N	HY+, ALH/ACD	D	D	HY+, ALH/ACD	N	HY+, ALH/ACD	S	N	D	
127	K	K	K	K	K	K	K	K	K	K	K	R	BAS	K	R		
128	P	P	P	P	P	P	P	P	P	P	P	S	ALP/ALH	P	S		
129	D	D	D	D	D	D	K	HY+, ACD/BAS	N	N	HY+, ACD/BAS		HY+, ACD/BAS OR GAP	D	N	K	
130	F	F	F	F	F	F	F	F	F	F	F		F OR GAP	F			
131	S	S	S	S	S	S	S	S	F	F	ALH/ARO		ALH/ARO OR GAP	S	F		
132	P	P	P	P	P	P	P	P	G	G	ALP		ALP OR GAP	P	G		
133	I	I	I	I	I	I	V	ALP	D	D	ALP/ACD		ALP/ACD OR GAP	I	D	V	
134	D	D	D	D	D	D	D	D	L	L	ACD/ALP		ACD/ALP OR GAP	D	L		
135									F	F	F, OR GAP		F, OR GAP	F			
136									V	V	V, OR GAP		V, OR GAP	V			

Fig. 7.3 cont.

Fig. 8.0

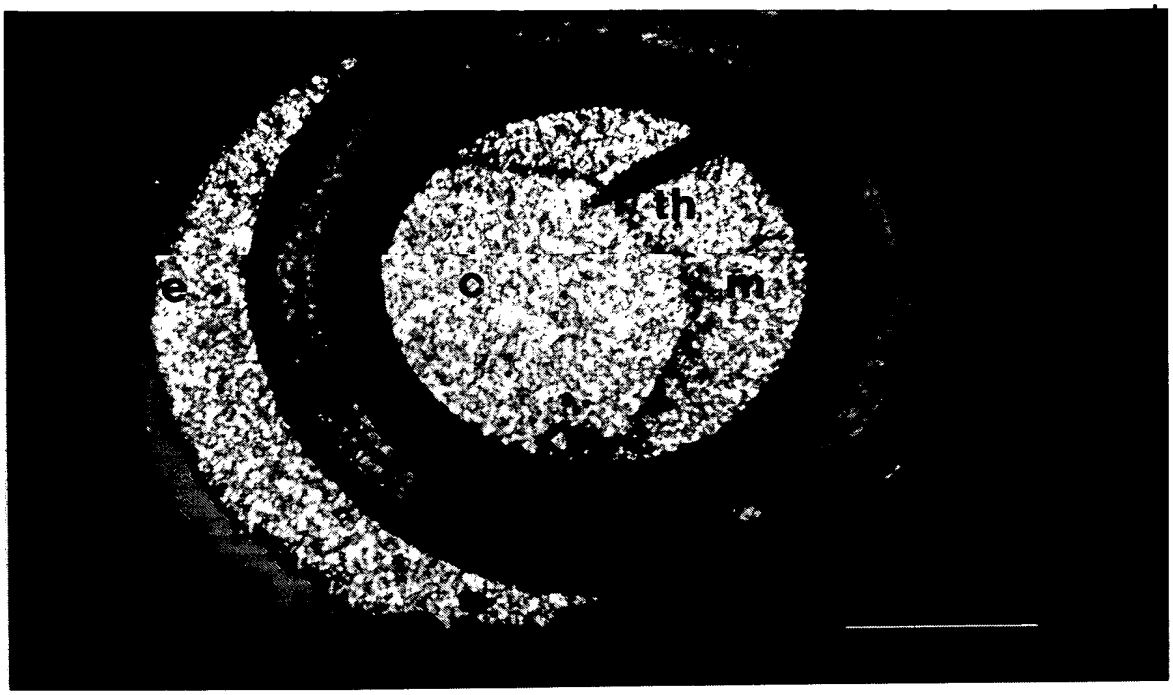


Fig. 8.1a

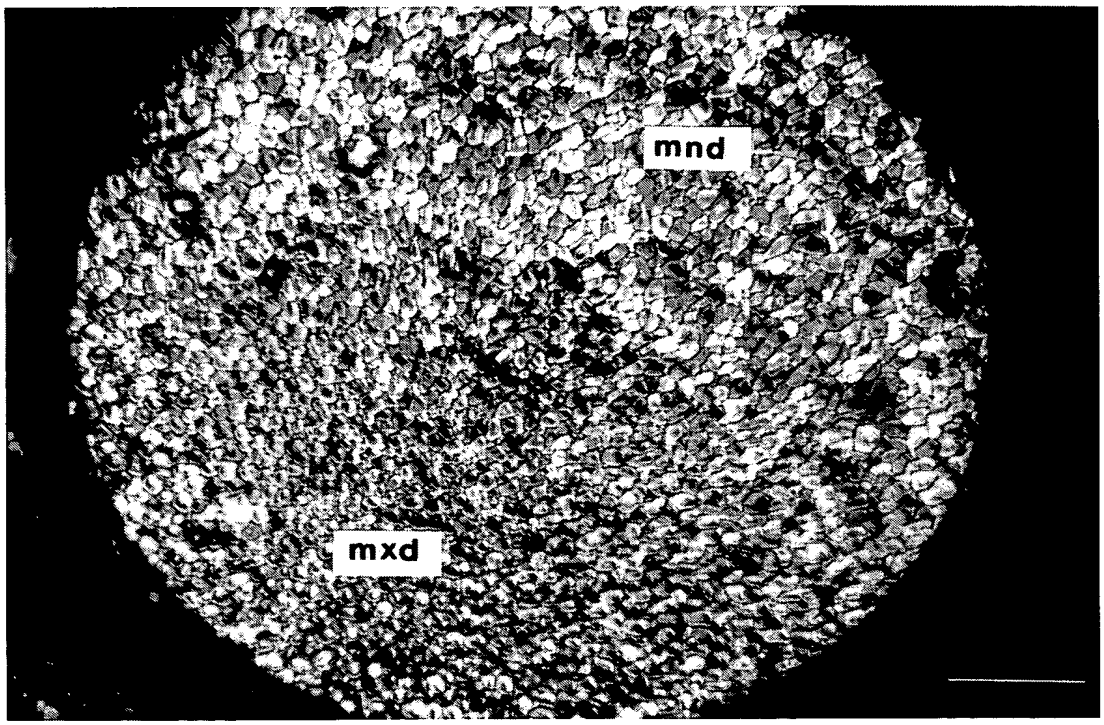
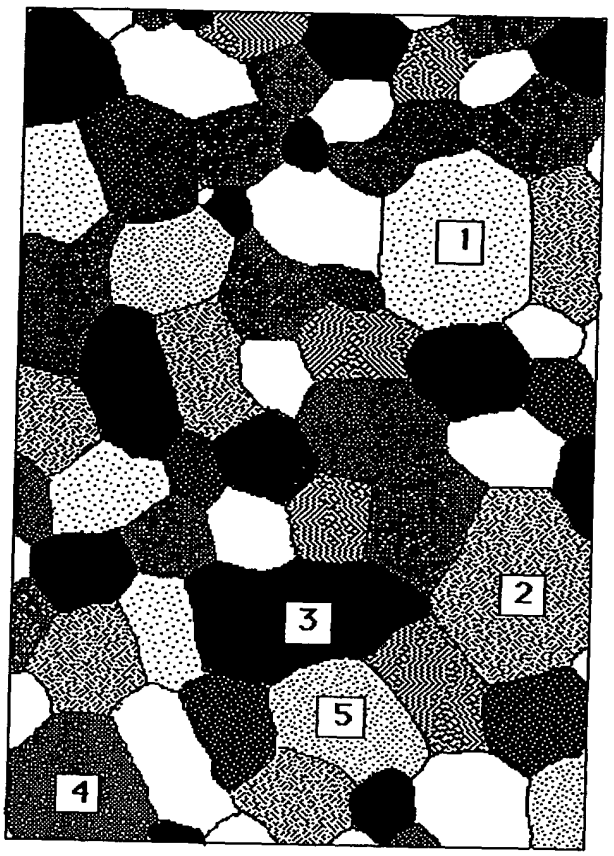
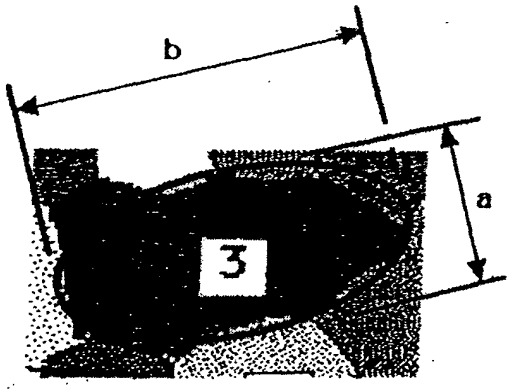


Fig. 8.1b





grain area=0.25πab

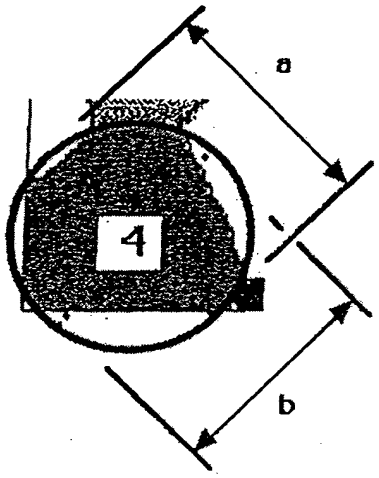


Fig. 8.2

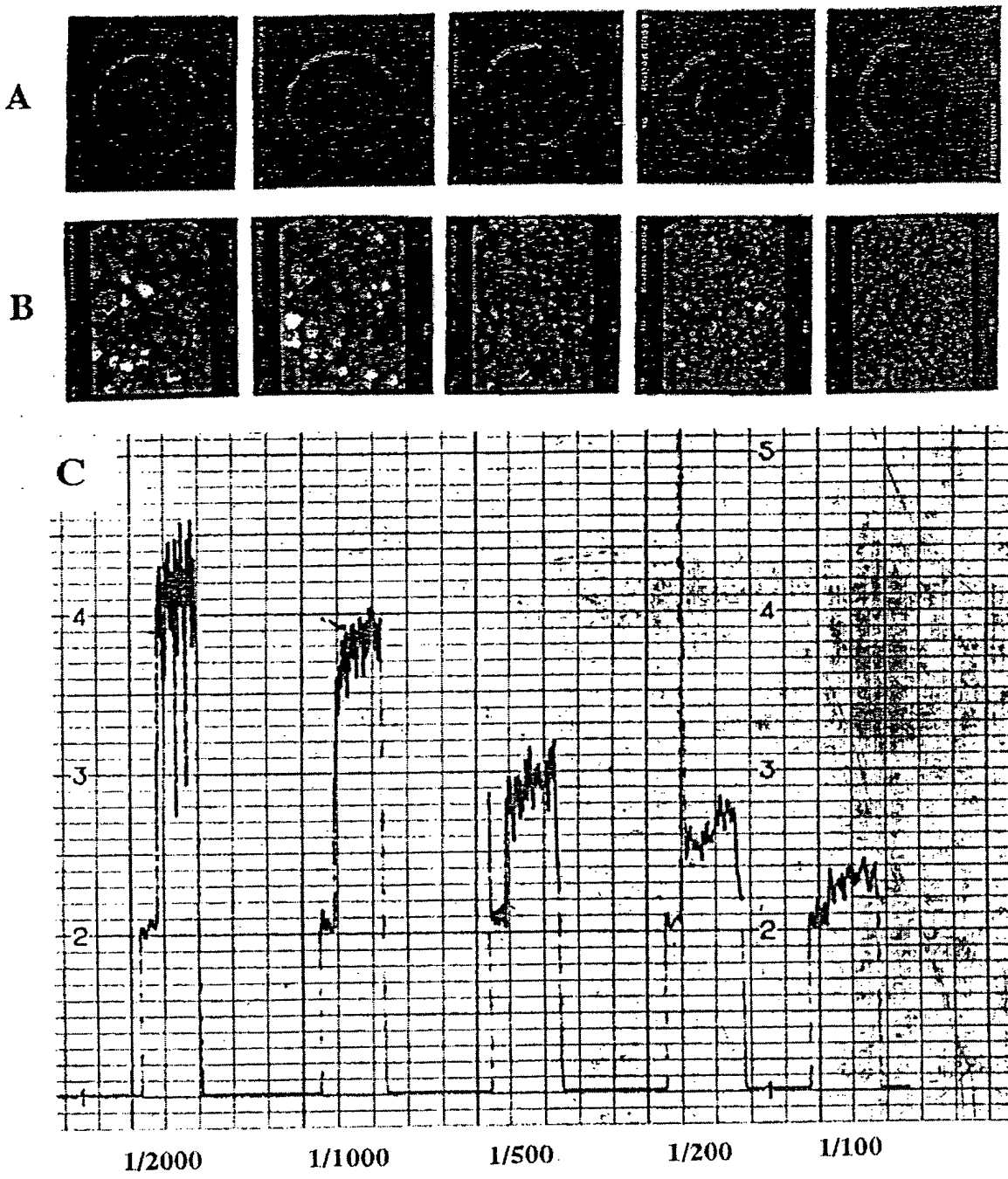


Fig. 8.3

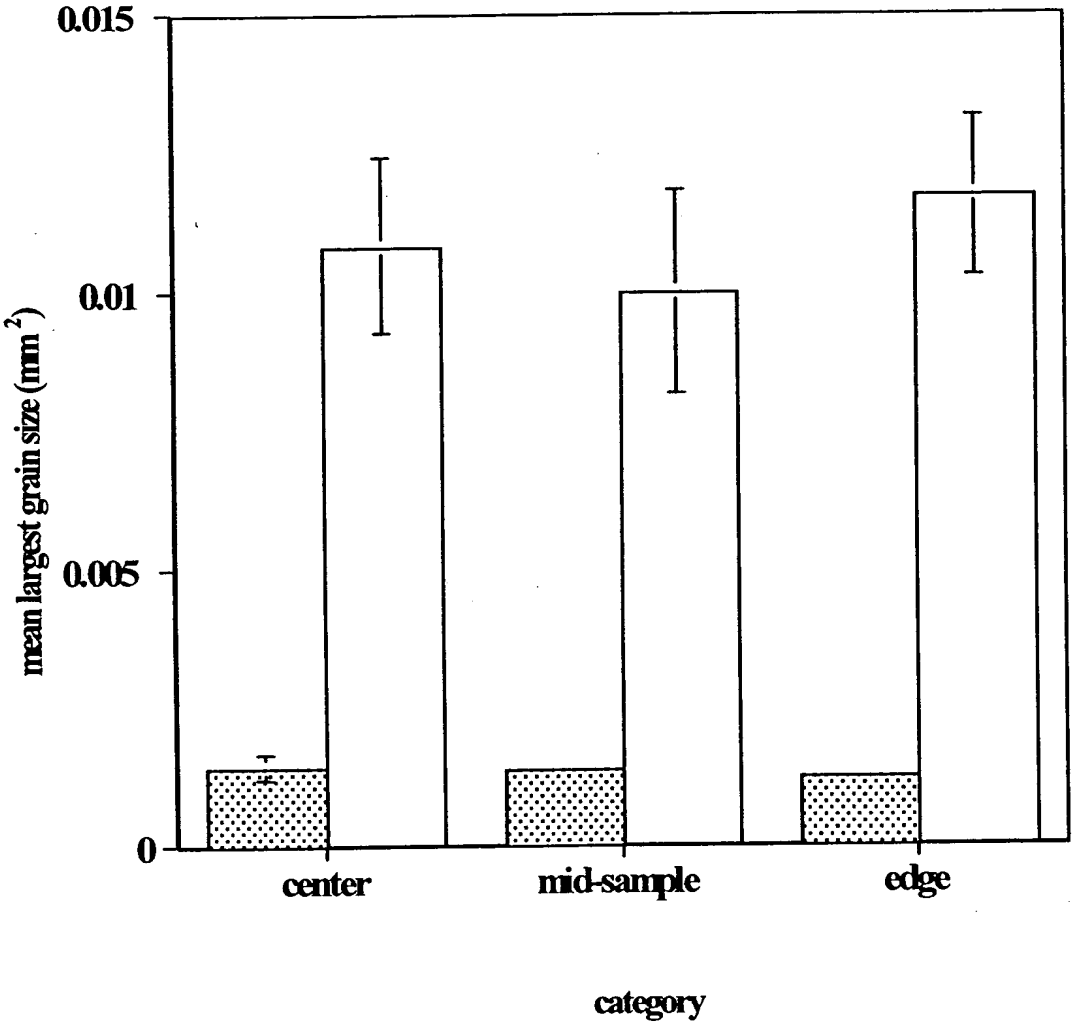

0.015
0.01
0.005
0
mean largest grain size (mm^2)
center
mid-sample
edge
category

Fig. 8.4a

0960-0360-0240-0202

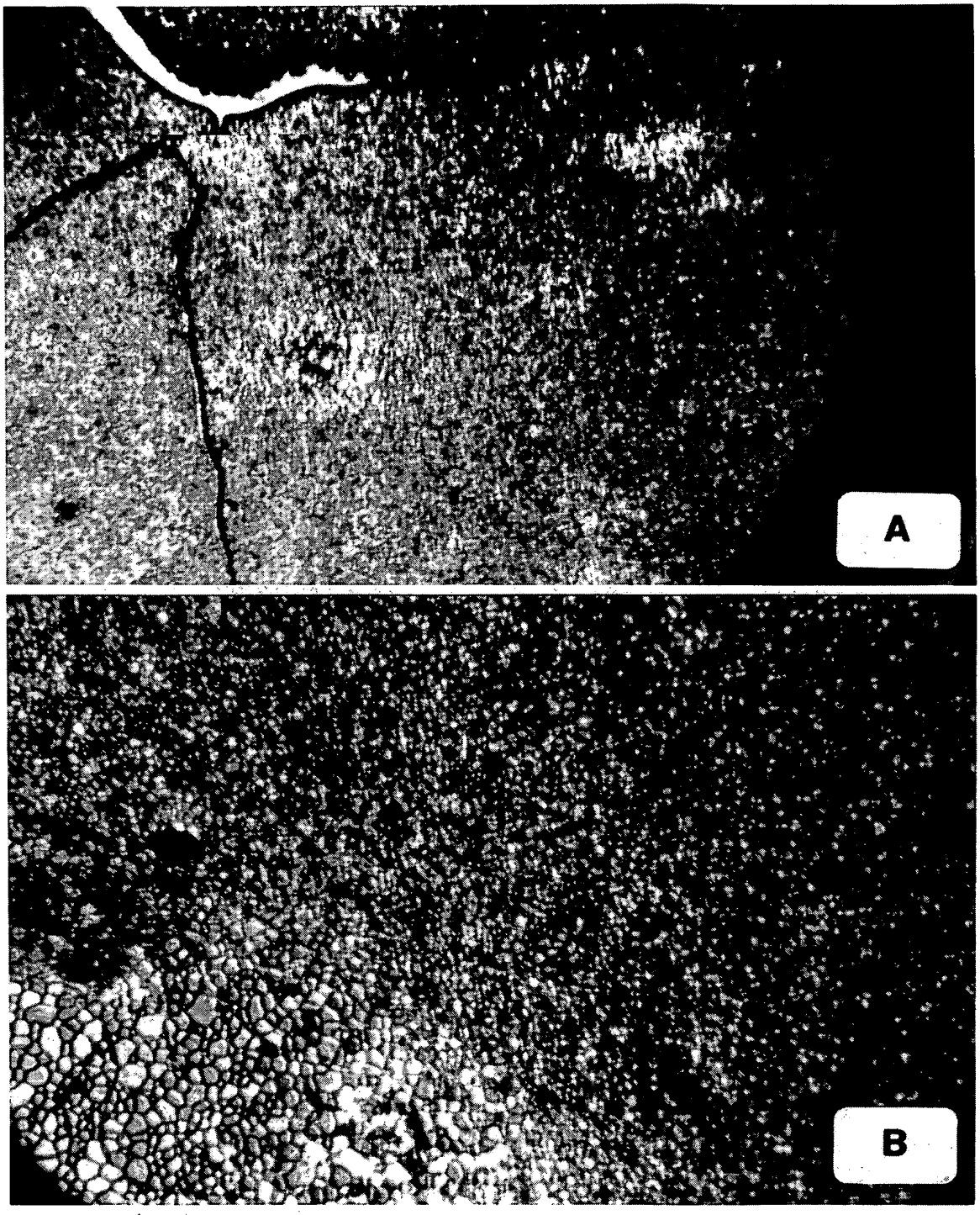


Fig. 8.4b

Case 29-95-2959

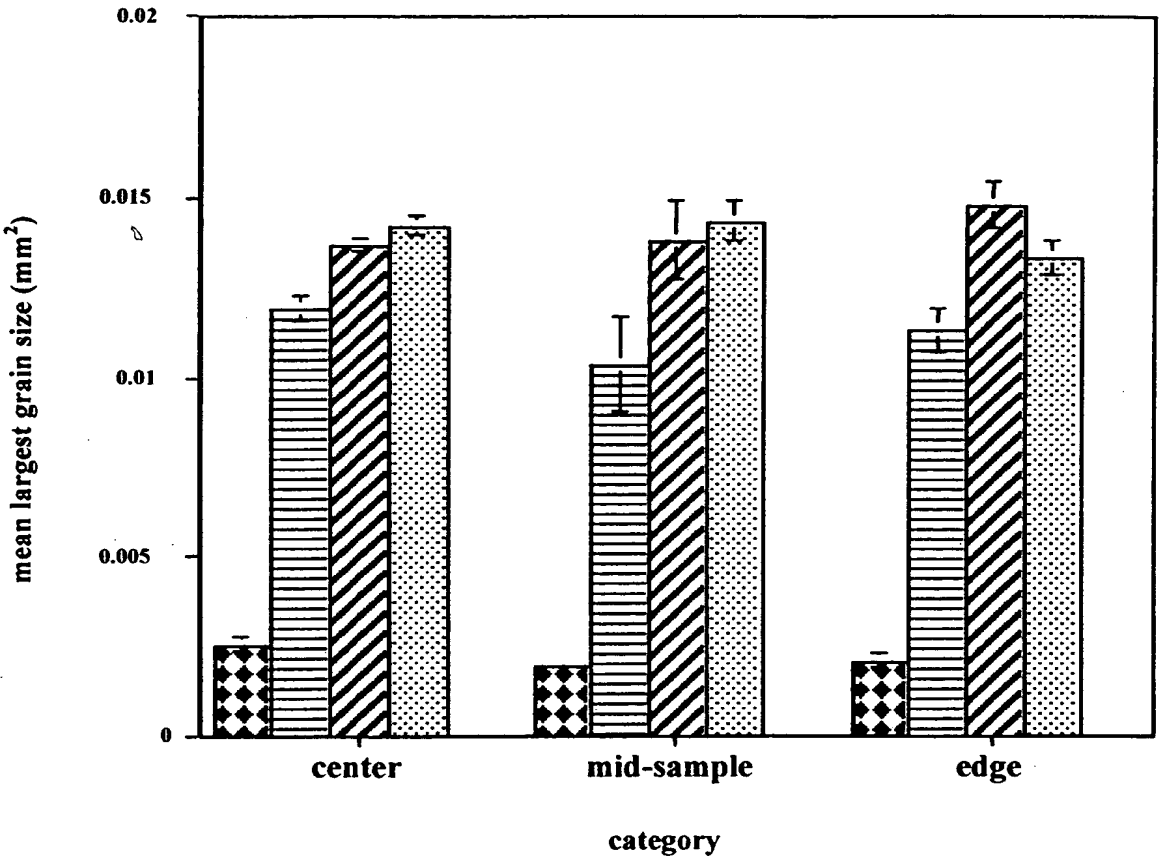


Fig. 8.5a

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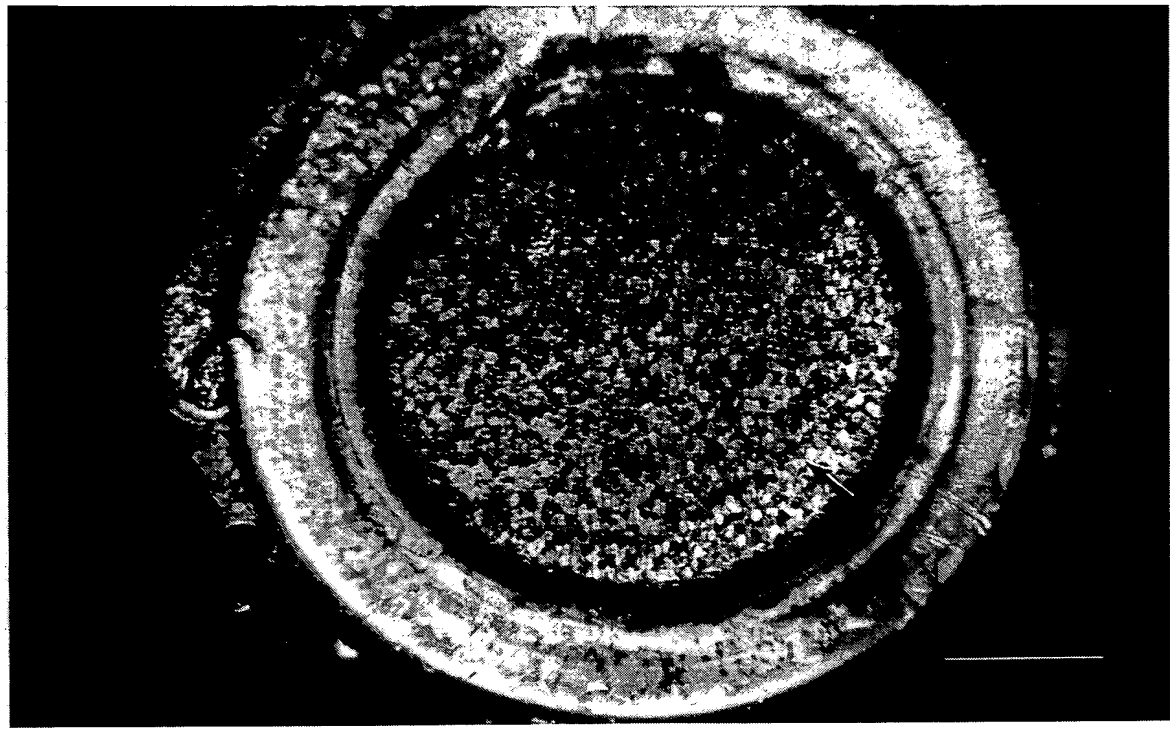

Fig. 8.5b

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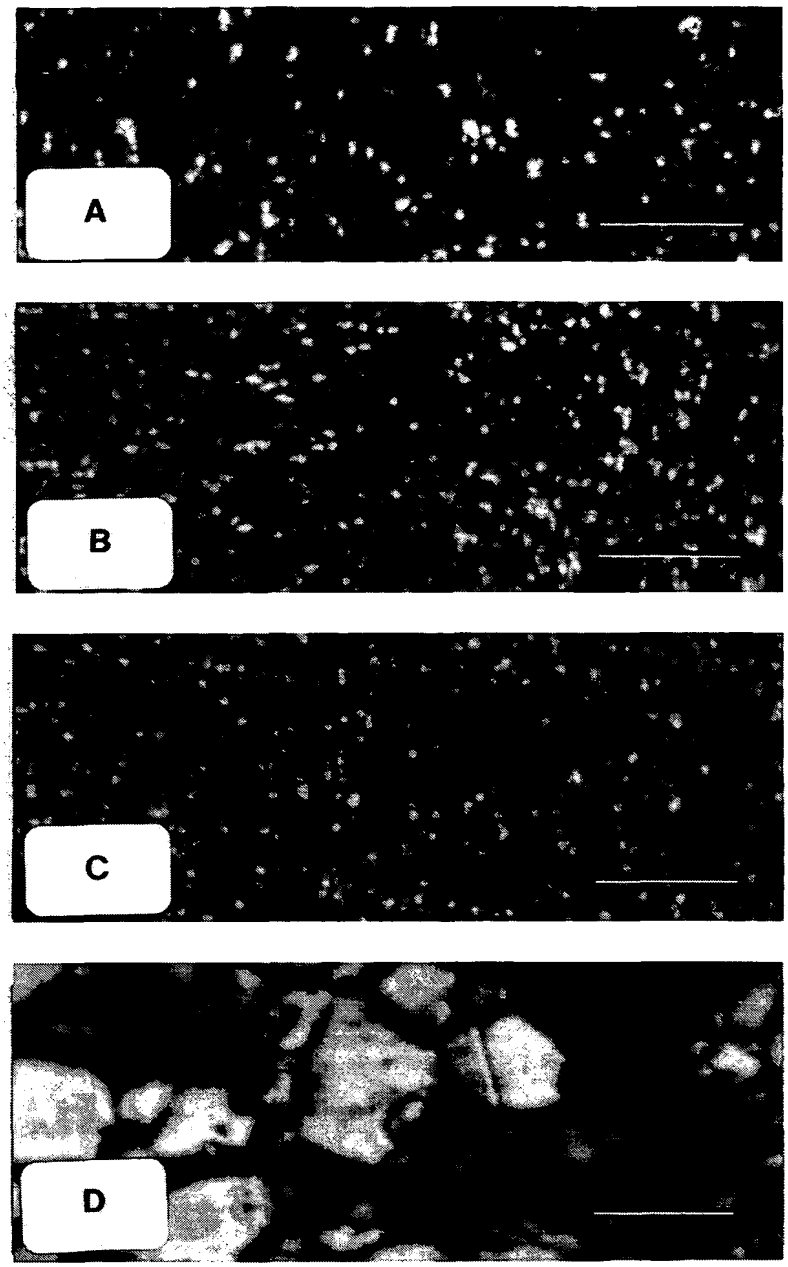

Fig. 8.6

0 3 0 3 7 0 3 0 1 0 3 0 4 5 0 2

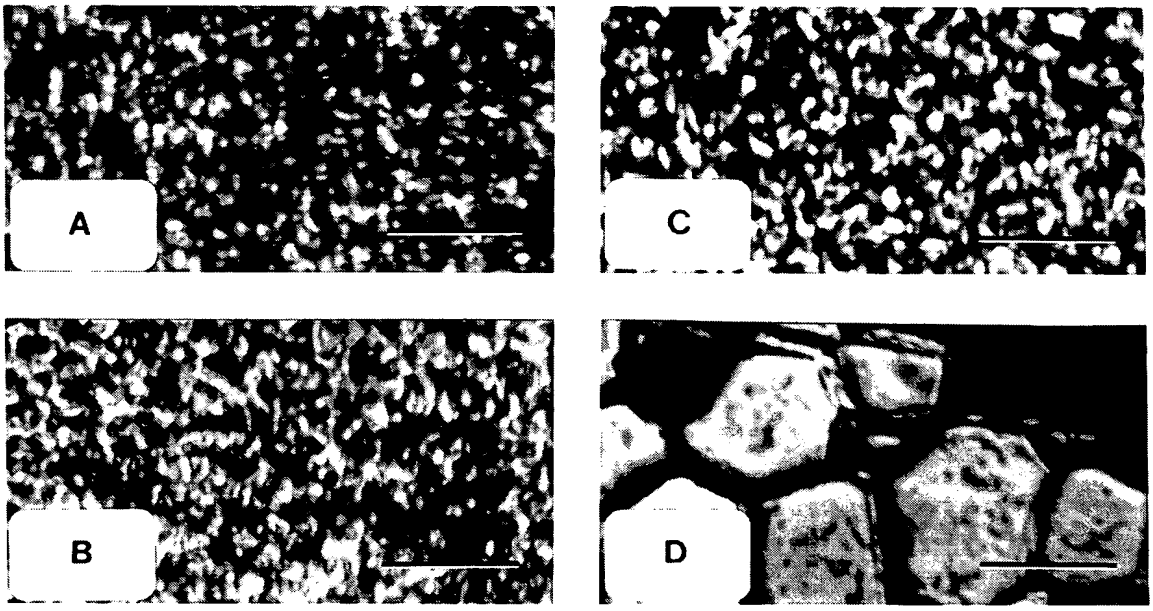

Fig. 8.7

20020730 960959

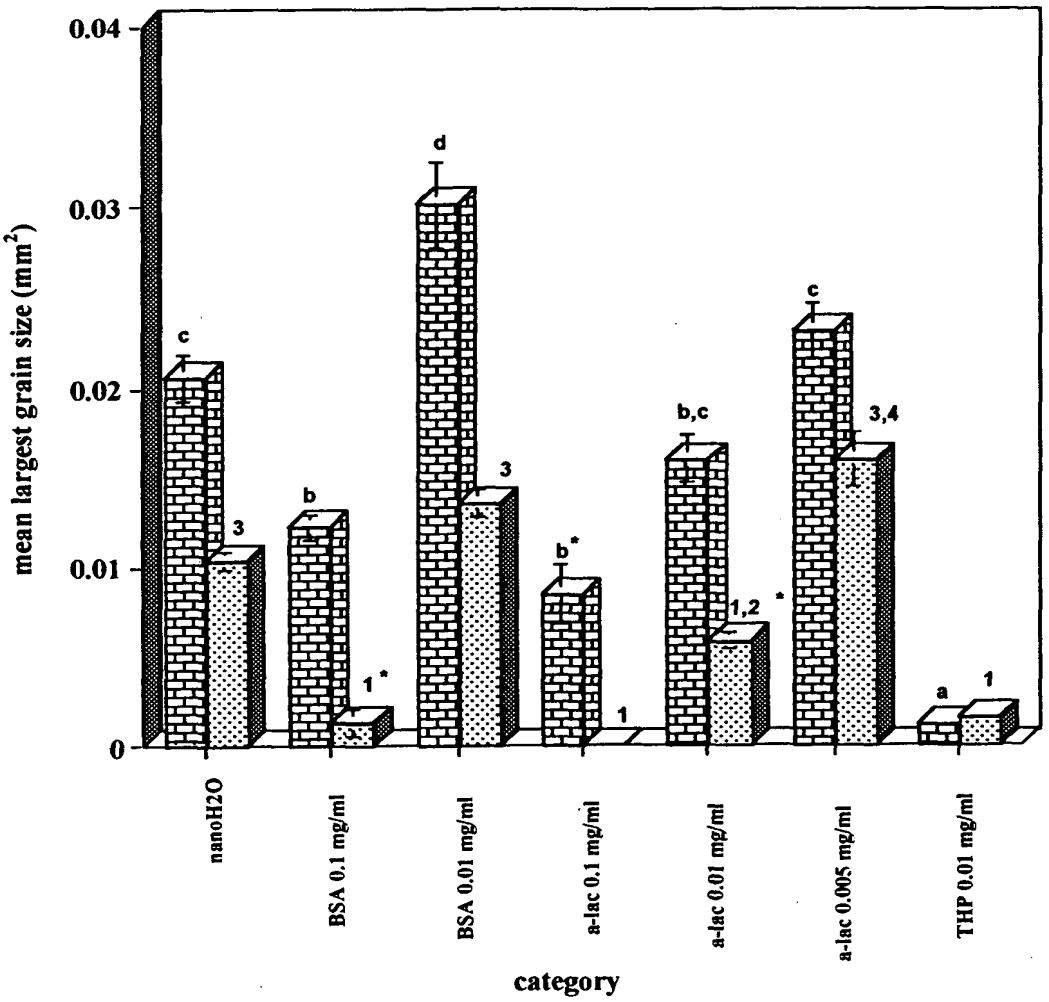

Fig. 8.8

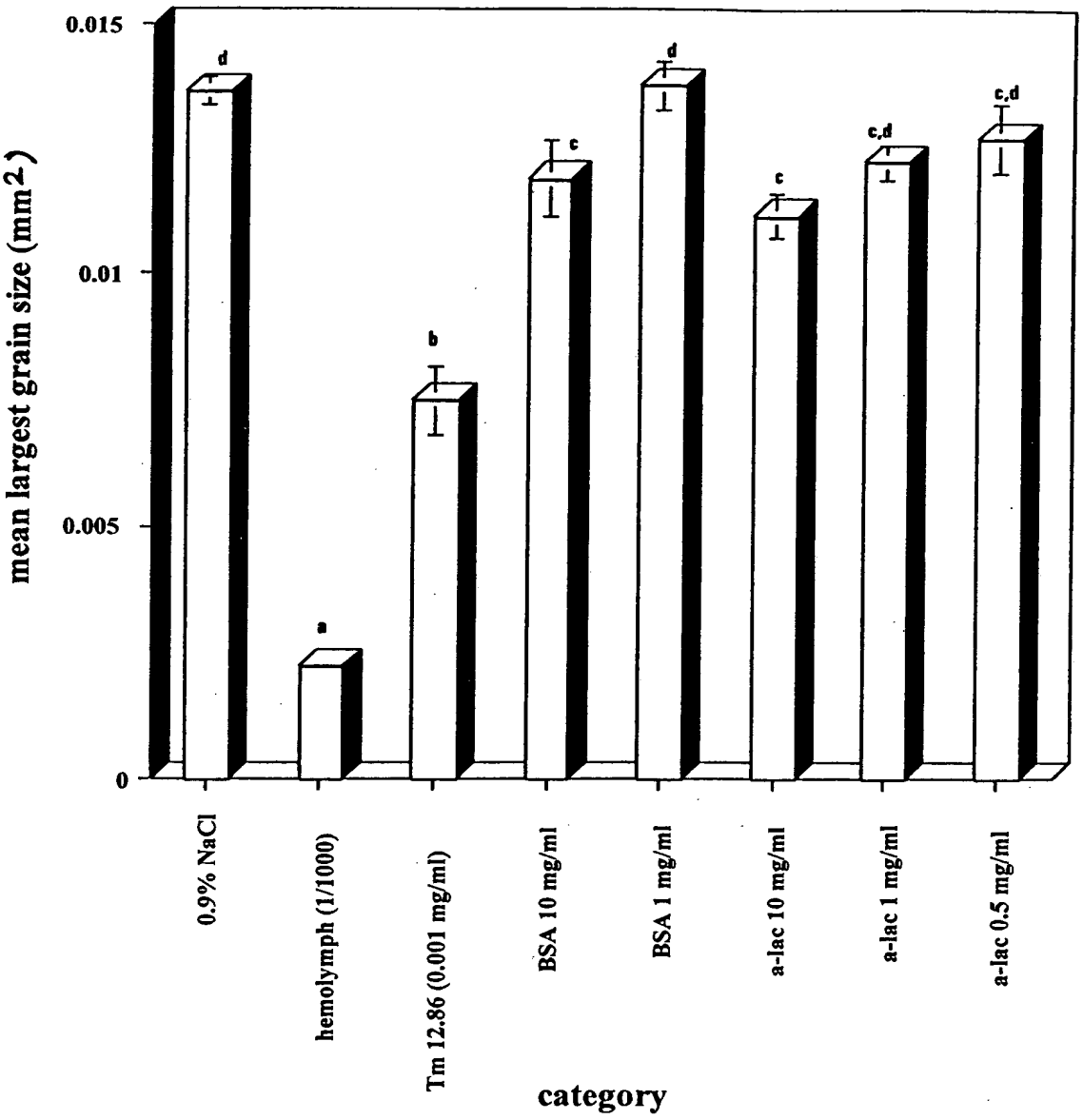
mean largest grain size (mm2)
0.015
0.01
0.005
0
d
a
b
c
d
c
c,d
c,d
0.9% NaCl
hemolymph (1/1000)
Tm 12.86 (0.001 mg/ml)
BSA 10 mg/ml
BSA 1 mg/ml
a-lac 10 mg/ml
a-lac 1 mg/ml
a-lac 0.5 mg/ml
category

Fig. 8.9

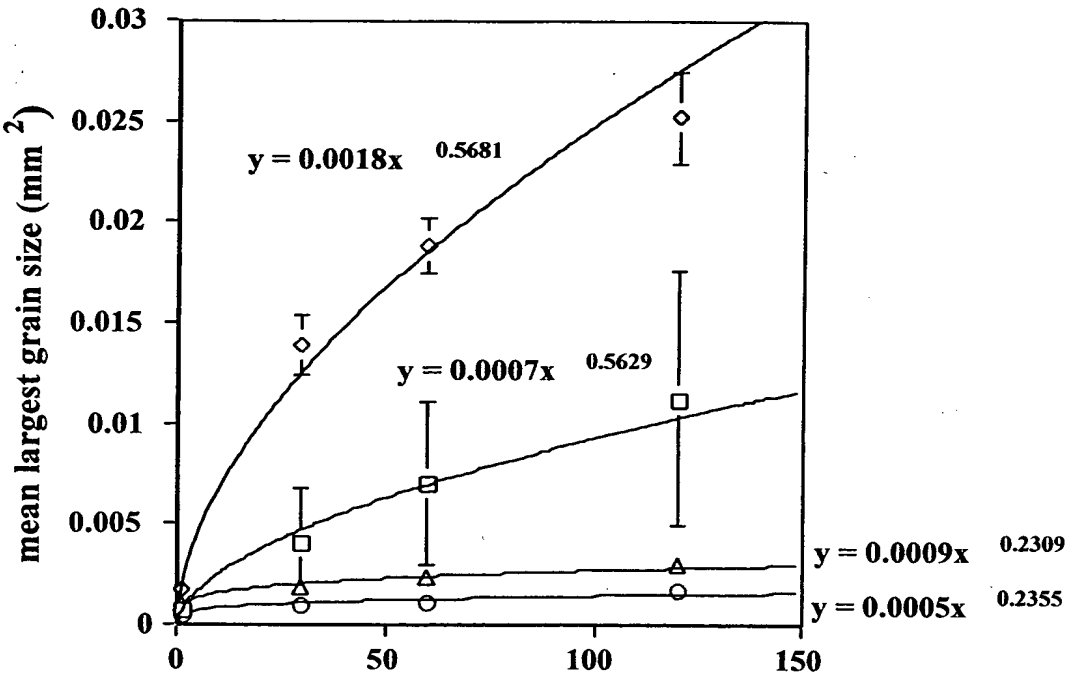
0.03
0.025
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
y = 0.0018x 0.5681
y = 0.0007x 0.5629
y = 0.0009x 0.2309
y = 0.0005x 0.2355
0
50
100
150
time (minutes)

Fig. 8.10

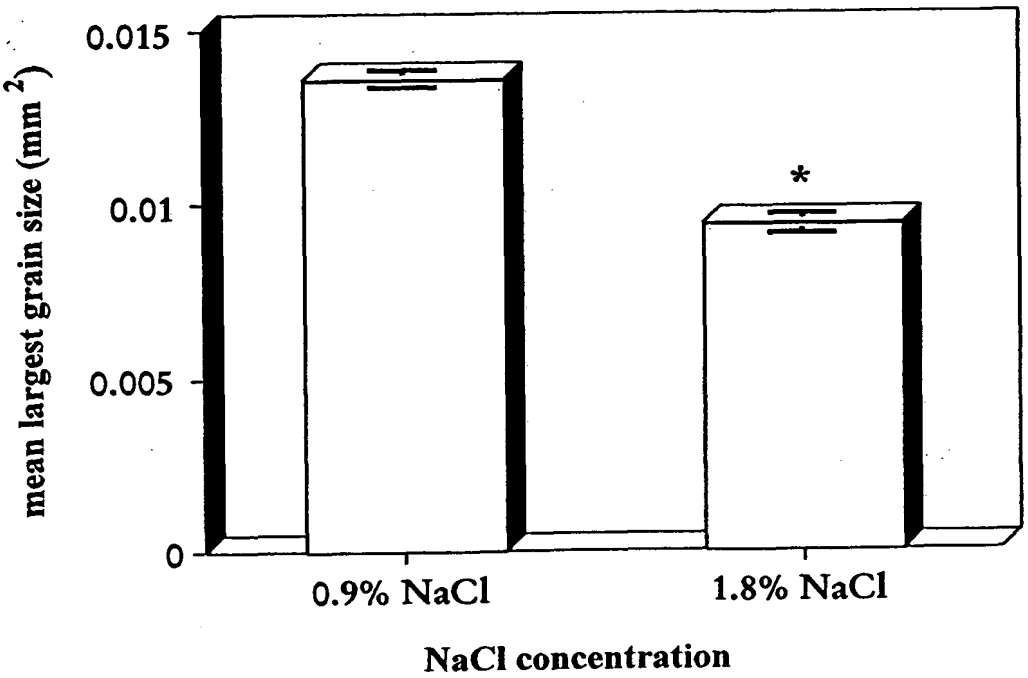
0.015
0.01
0.005
0
mean largest grain size (mm 2)
*
0.9% NaCl
1.8% NaCl
NaCl concentration

Fig. 8.11

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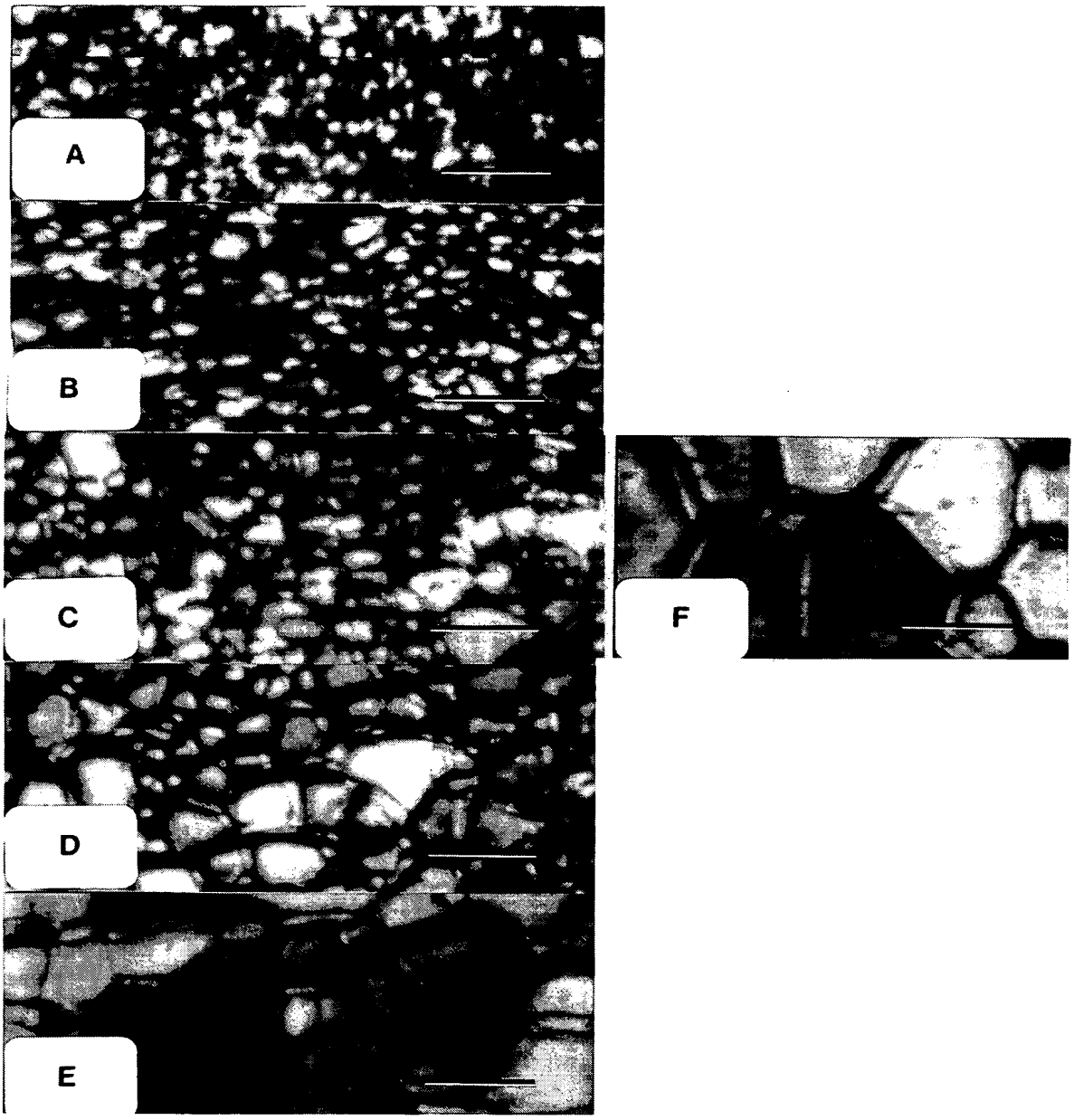


Fig. 8.12

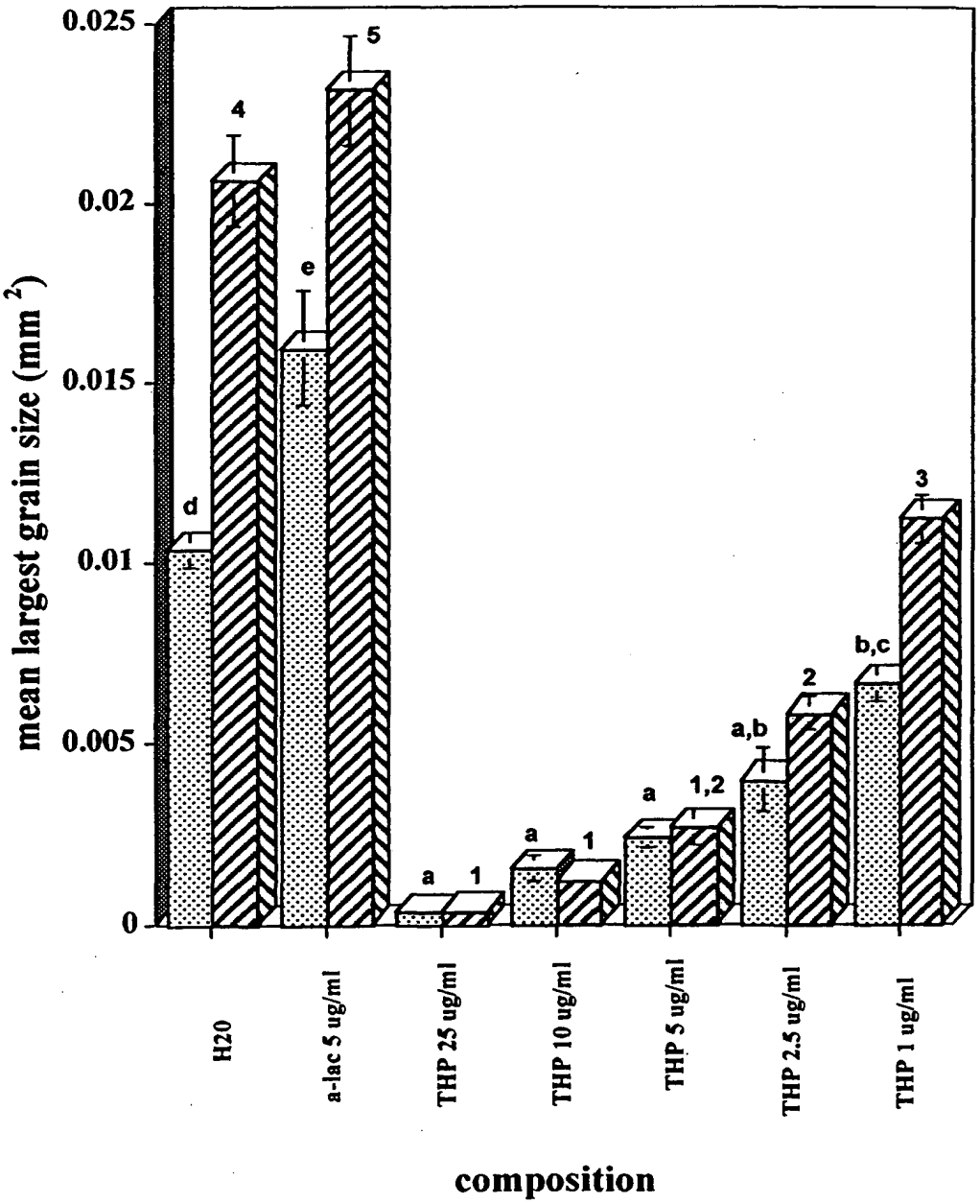

0.025
0.02
0.015
0.01
0.005
0
mean largest grain size (mm 2)
d
4
e
5
a
1
a
1
a
1,2
a,b
2
b,c
3
H20
a-lac 5 ug/ml
THP 25 ug/ml
THP 10 ug/ml
THP 5 ug/ml
THP 2.5 ug/ml
THP 1 ug/ml
composition

Fig. 8.13

Fig. 8.14

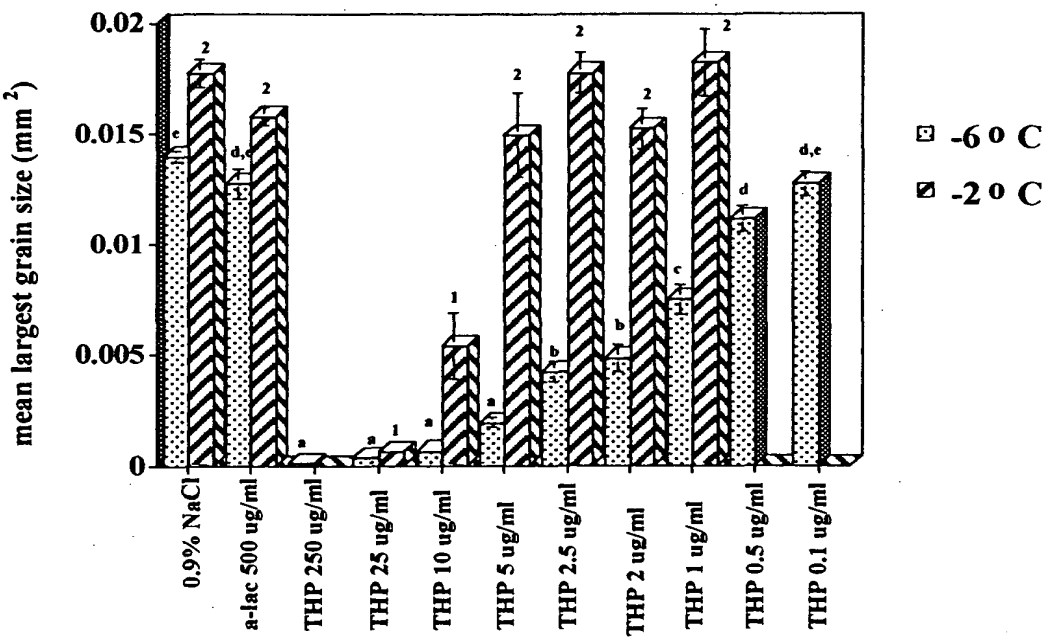
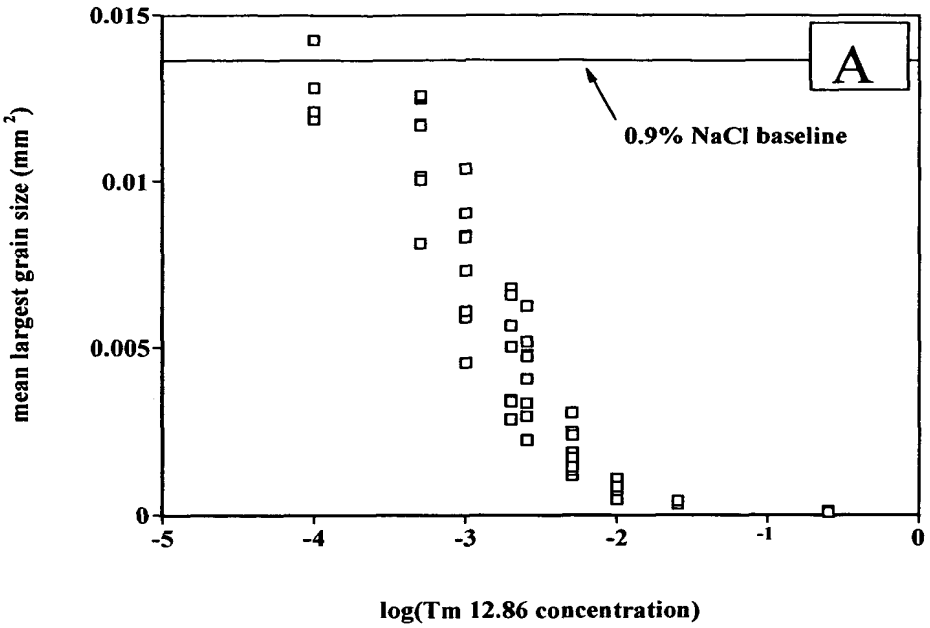

mean largest grain size (mm 2)
0.02
0.015
0.01
0.005
0
0.9% NaCl
a-lac 500 ug/ml
THP 250 ug/ml
THP 25 ug/ml
THP 10 ug/ml
THP 5 ug/ml
THP 2.5 ug/ml
THP 2 ug/ml
THP 1 ug/ml
THP 0.5 ug/ml
THP 0.1 ug/ml
-6 o C
-2 o C

Fig. 8.15


A
0.9% NaCl baseline
mean largest grain size (mm 2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
0
log(Tm 12.86 concentration)

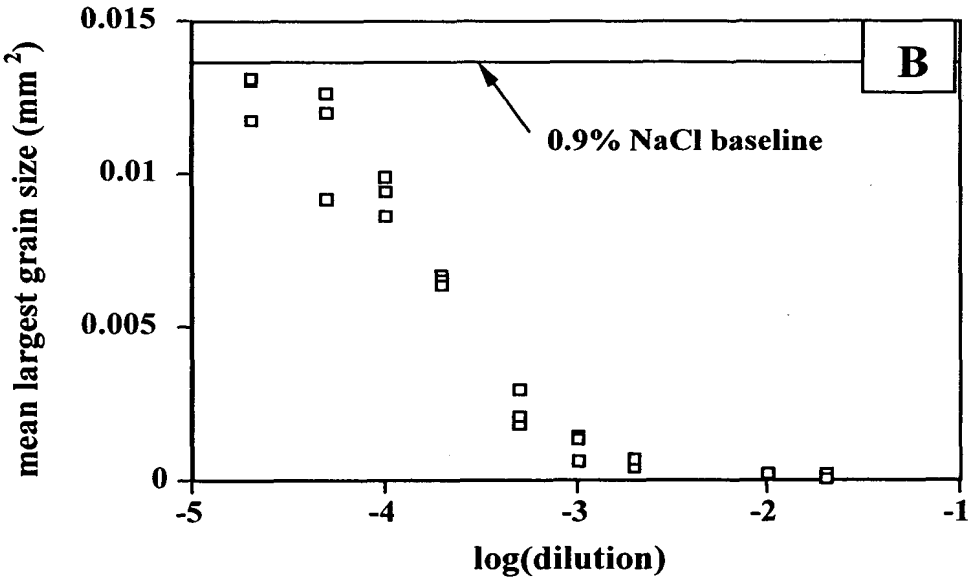
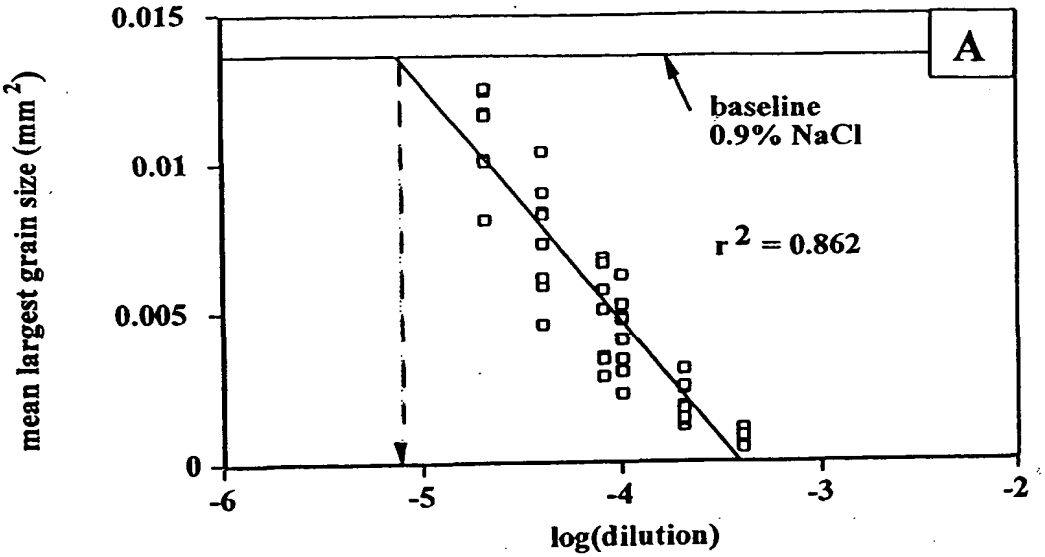

B
0.9% NaCl baseline
mean largest grain size (mm 2)
0.015
0.01
0.005
0
-5
-4
-3
-2
-1
log(dilution)

Fig. 8.16


A
baseline
0.9% NaCl
$r^2 = 0.862$
mean largest grain size (mm^2)
0.015
0.01
0.005
0
-6
-5
-4
-3
-2
log(dilution)

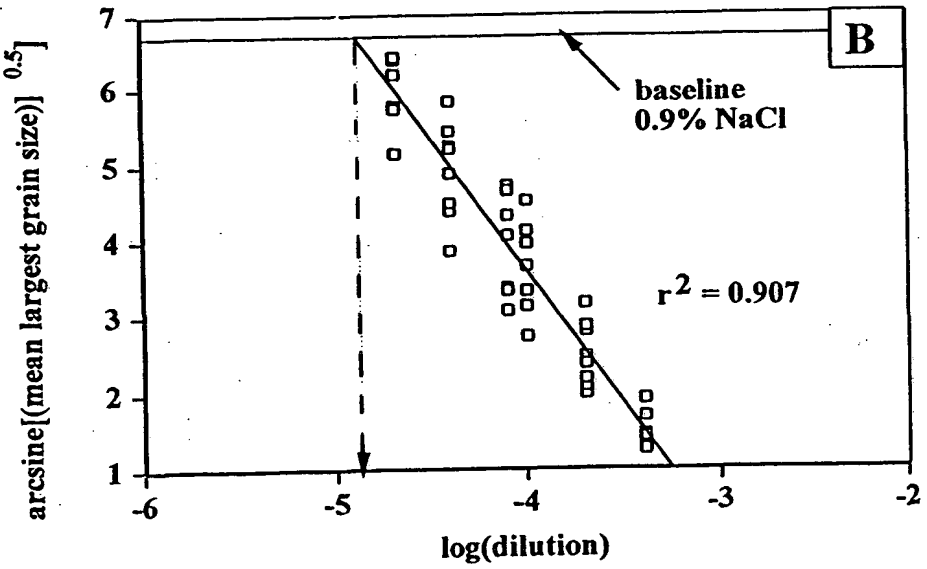

B
baseline
0.9% NaCl
$r^2 = 0.907$
arcsine[(mean largest grain size)] $^{0.5}$]
7
6
5
4
3
2
1
-6
-5
-4
-3
-2
log(dilution)

Fig. 8.17

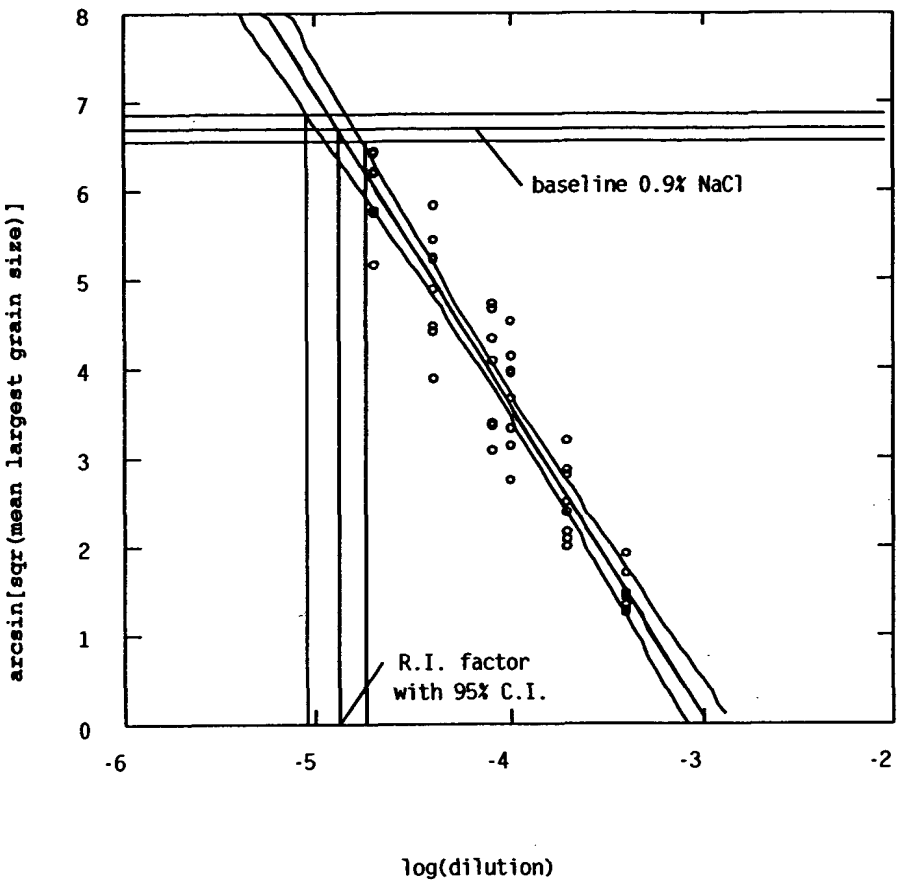

arcsin[sqr(mean largest grain size)]
log(dilution)
baseline 0.9% NaCl
R.I. factor
with 95% C.I.
8
7
6
5
4
3
2
1
0
-6
-5
-4
-3
-2

Fig. 8.18

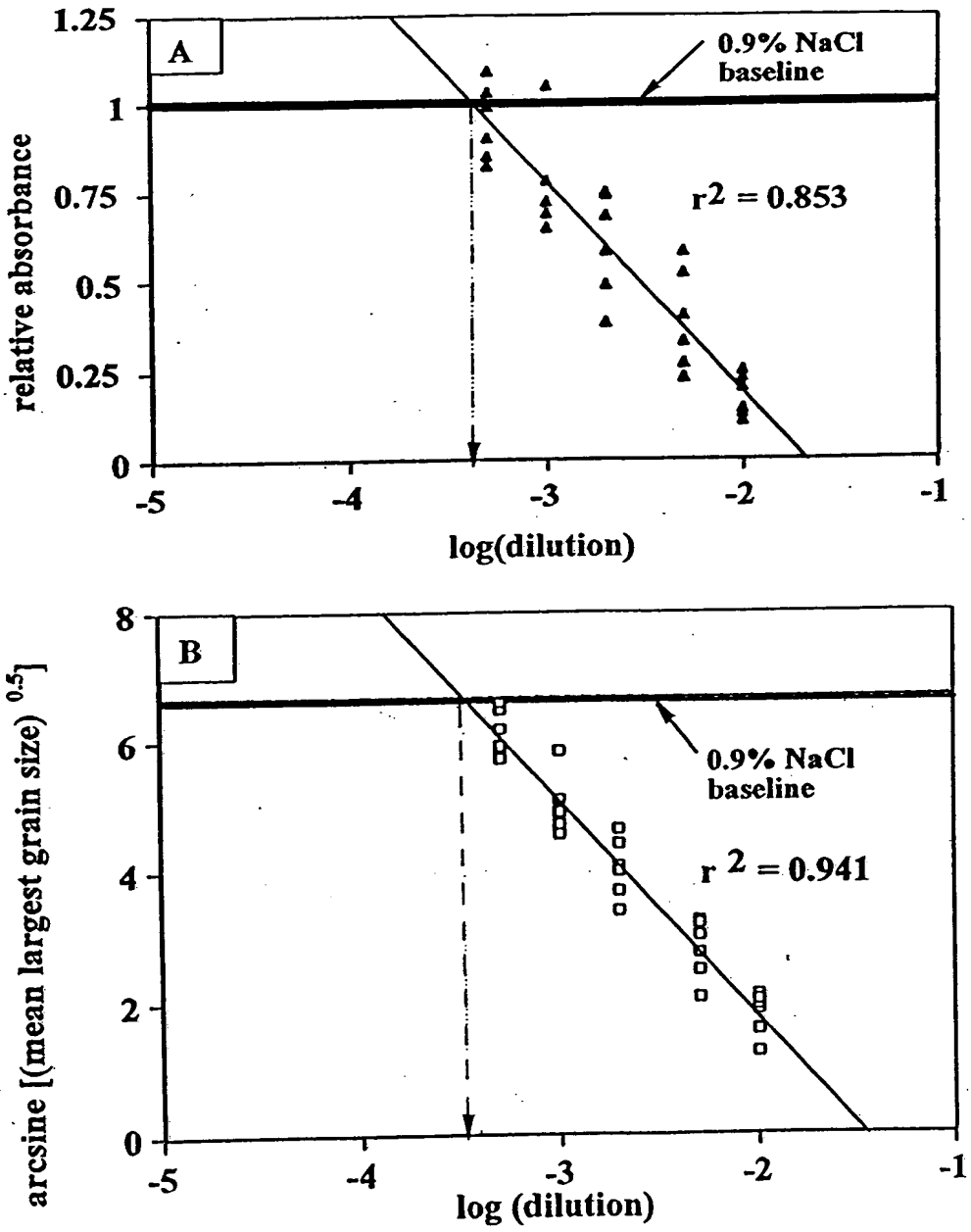

A
0.9% NaCl baseline
$r^2 = 0.853$
relative absorbance
1.25
1
0.75
0.5
0.25
0
-5
-4
-3
-2
-1
log(dilution)
B
0.9% NaCl baseline
$r^2 = 0.941$
arcsine [(mean largest grain size) $^{0.5}$]
8
6
4
2
0
-5
-4
-3
-2
-1
log (dilution)

Fig. 8.19

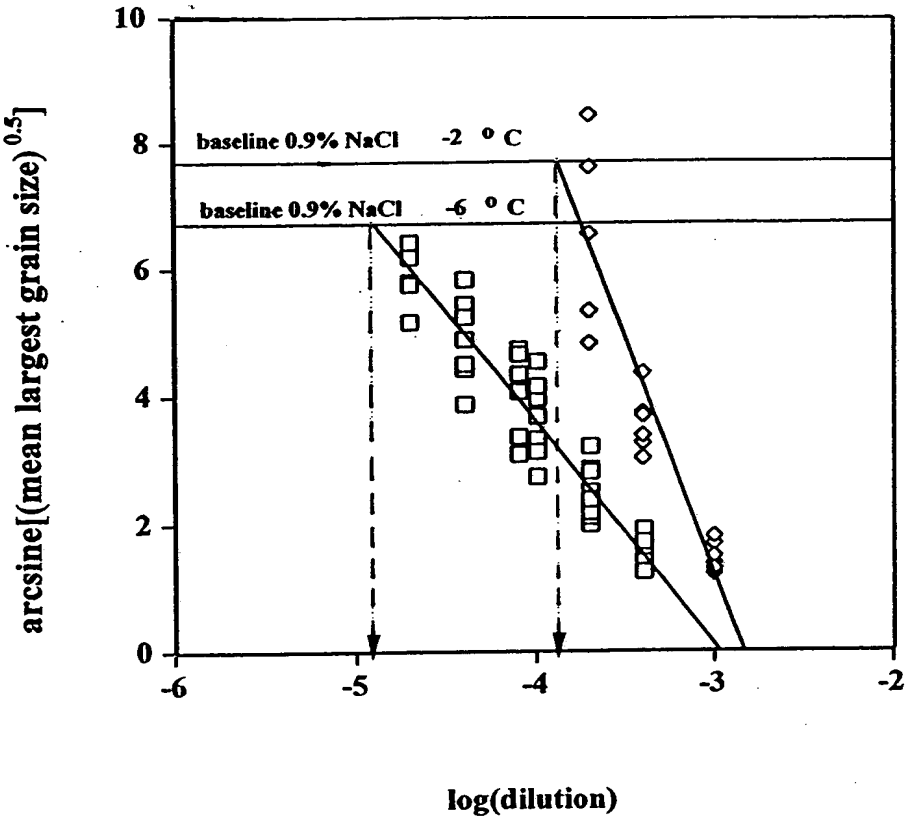

10
8
6
4
2
0
-6
-5
-4
-3
-2
baseline 0.9% NaCl -2 ° C
baseline 0.9% NaCl -6 ° C
arcsine[(mean largest grain size)^0.5]
log(dilution)

Fig. 8.20

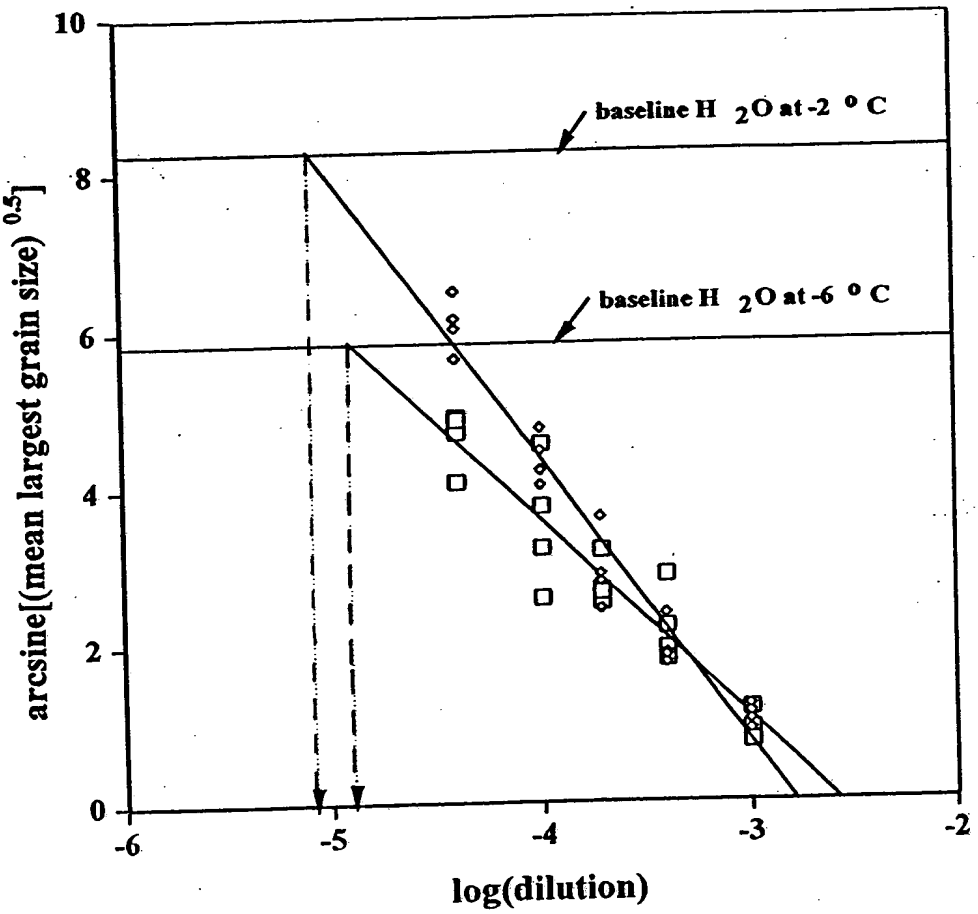

10
8
6
4
2
0
-6
-5
-4
-3
-2
baseline H 2O at -2 ° C
baseline H 2O at -6 ° C
arcsine[(mean largest grain size) 0.5]
log(dilution)

Fig. 8.21

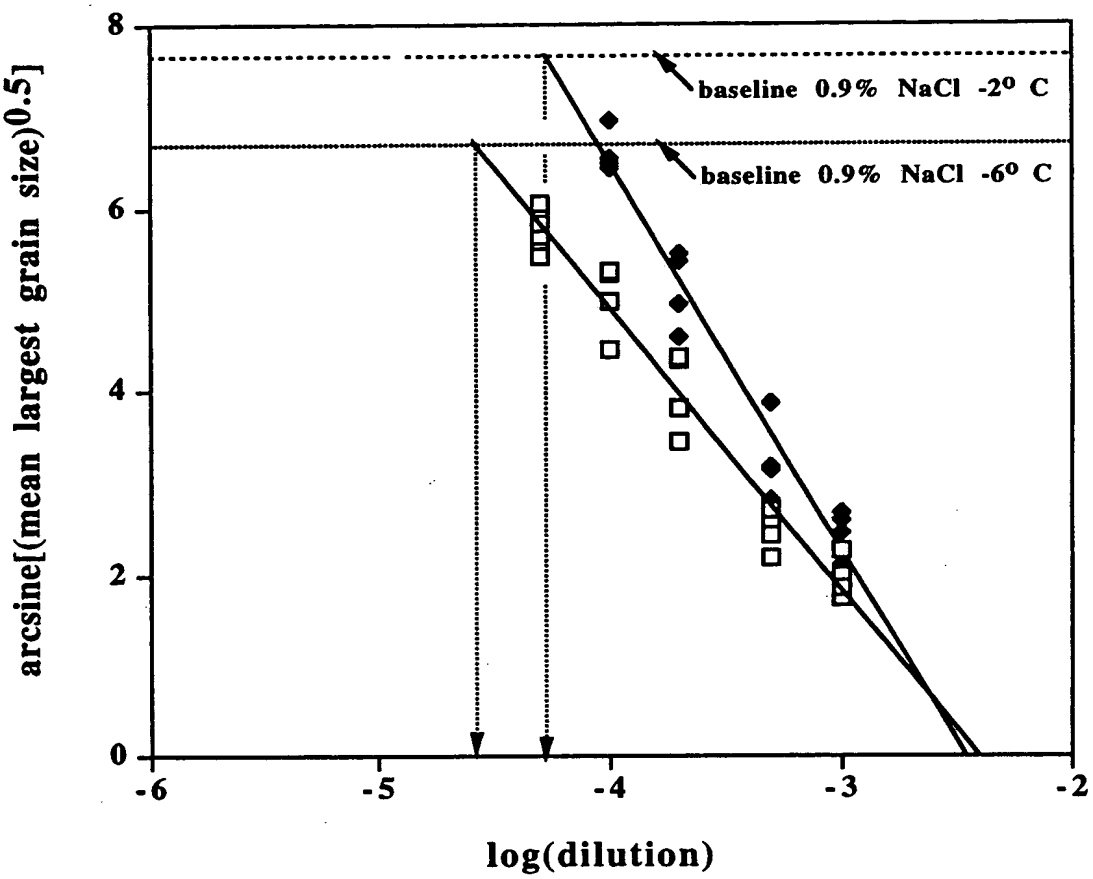

8
6
4
2
0
-6
-5
-4
-3
-2
arcsine[(mean largest grain size)0.5]
log(dilution)
baseline 0.9% NaCl -2° C
baseline 0.9% NaCl -6° C

Fig. 8.22

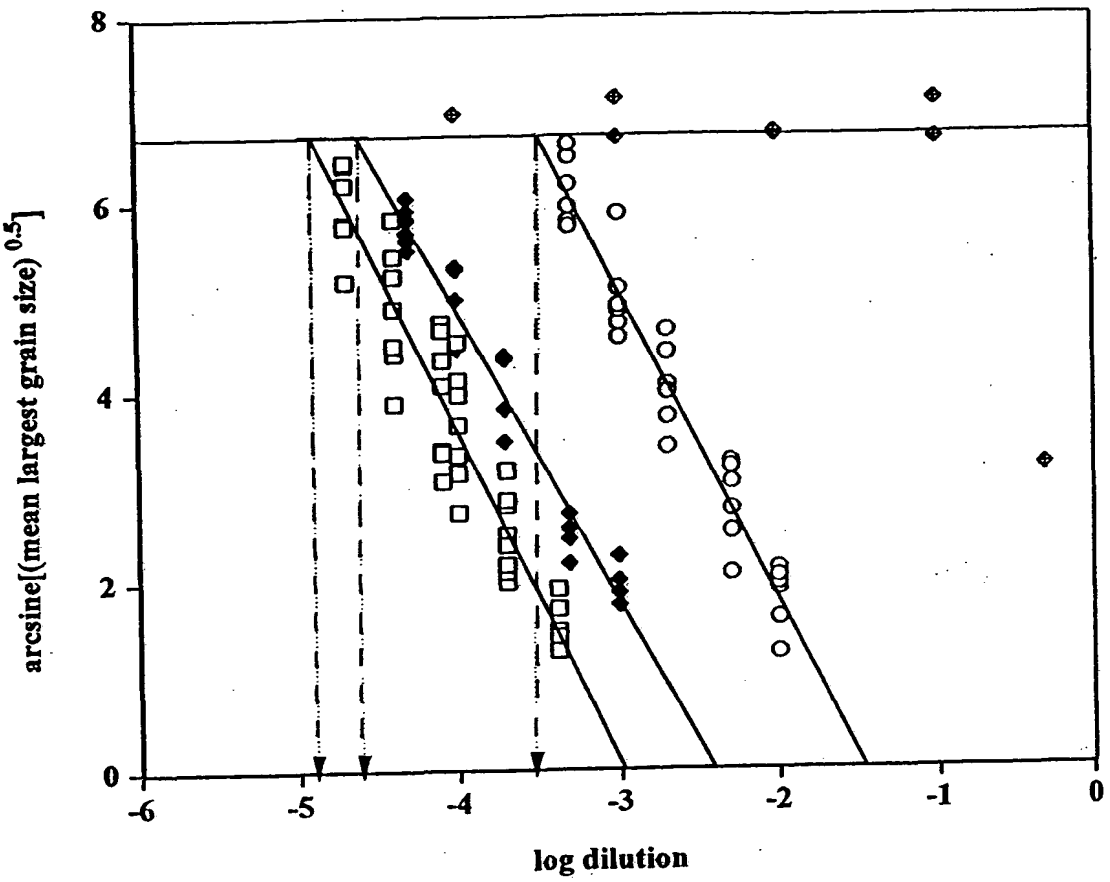

8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
arcsine[(mean largest grain size) 0.5]
log dilution

Fig. 8.23

25
10
5.0
2.5
1.0
0.5
0.25
0.1 mg/ml Tm 12.86
baseline 0.9% NaCl
arcsine[(mean largest grain size)0.5]
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
log dilution

Fig. 8.24

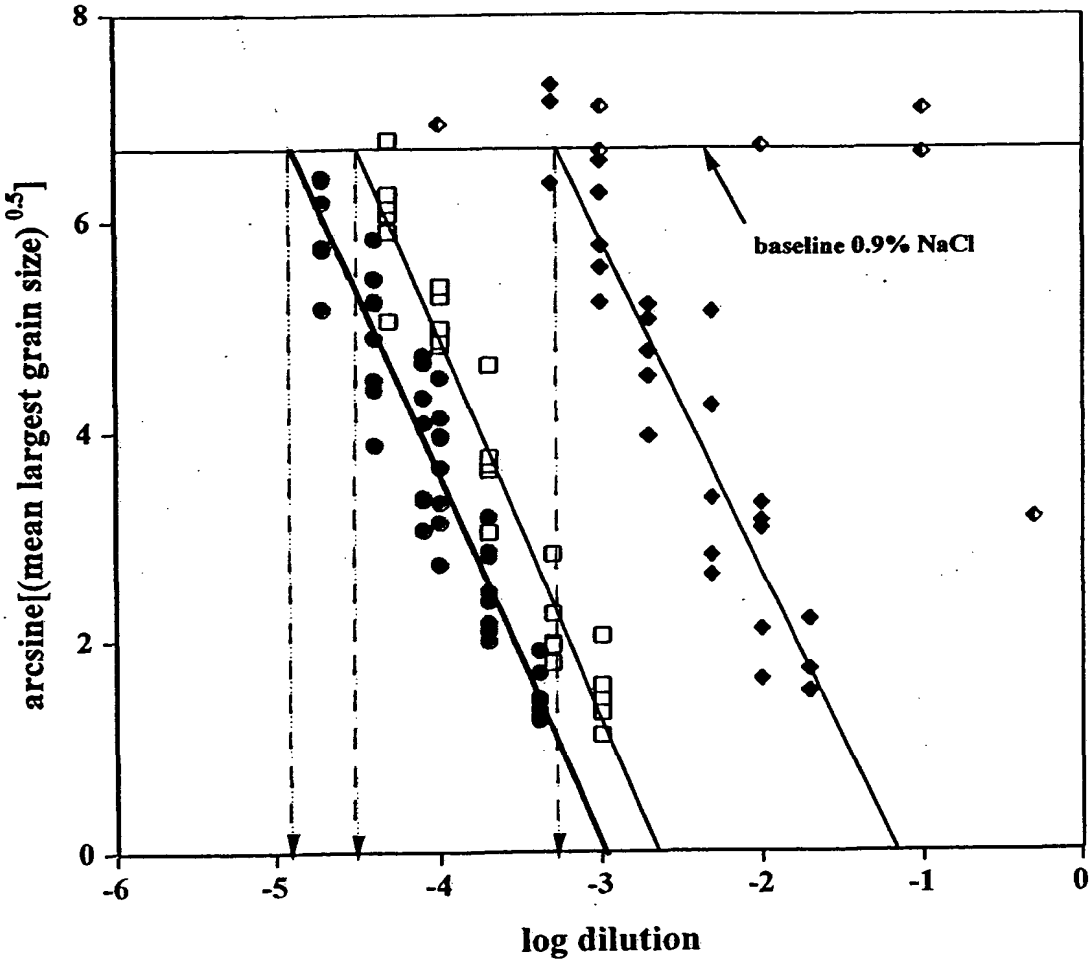

8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
arcsine[(mean largest grain size)^0.5]
log dilution
baseline 0.9% NaCl

Fig. 8.25

25
10
5.0
2.5
1.0
0.5
0.25
0.1 mg/ml Tm 12.86
0.9% NaCl baseline
arcsine[(mean largest grain size)0.5]
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
log dilution

Fig. 8.26

20220525205

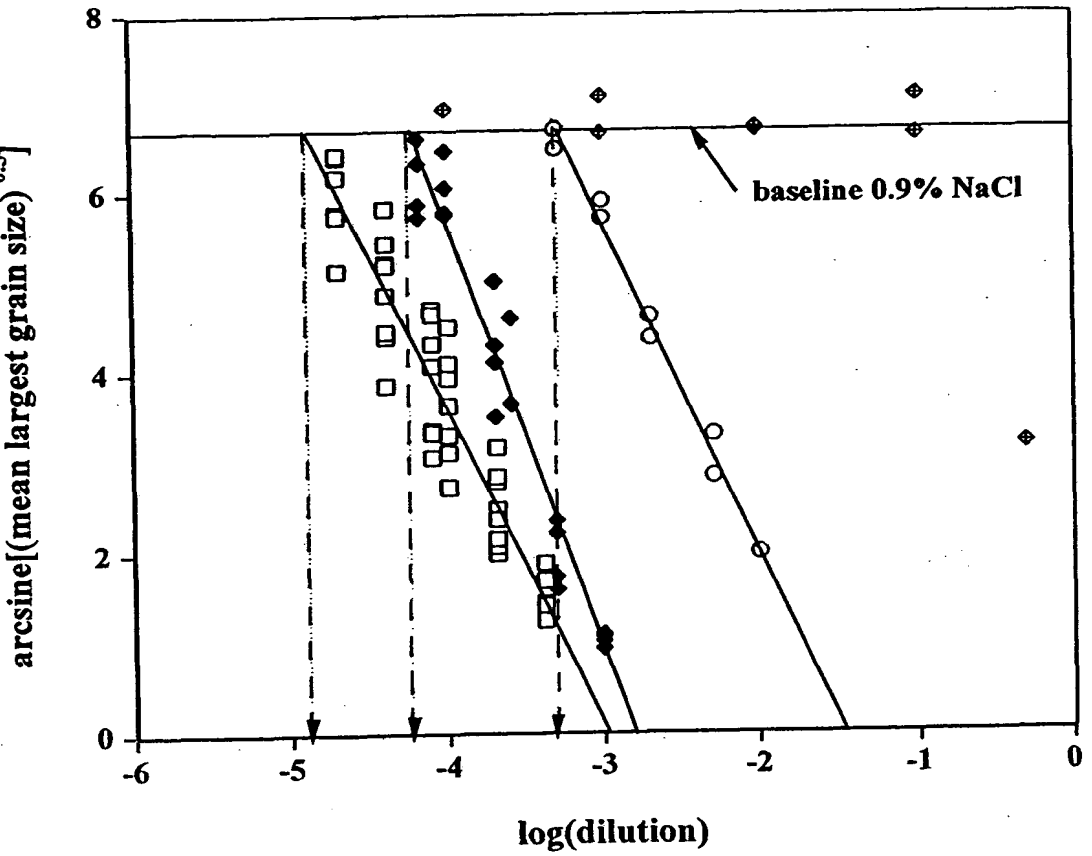


Fig. 8.27

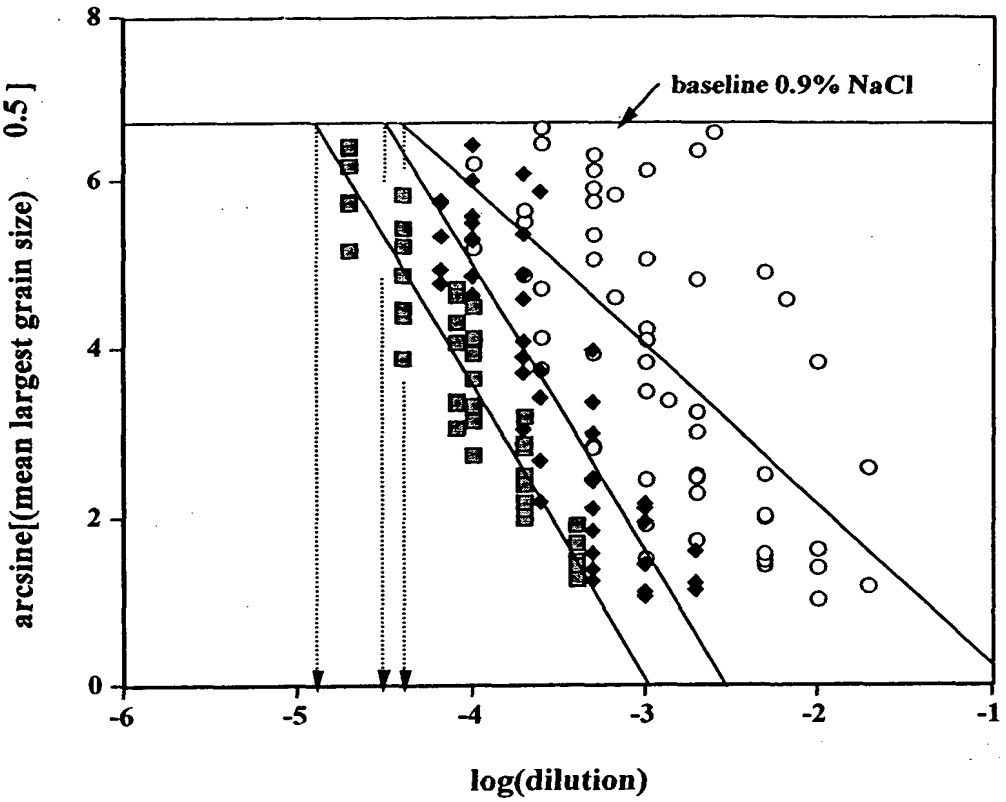

baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5]
log(dilution)
8
6
4
2
0
-6
-5
-4
-3
-2
-1

Fig. 8.28

20020820 05420050

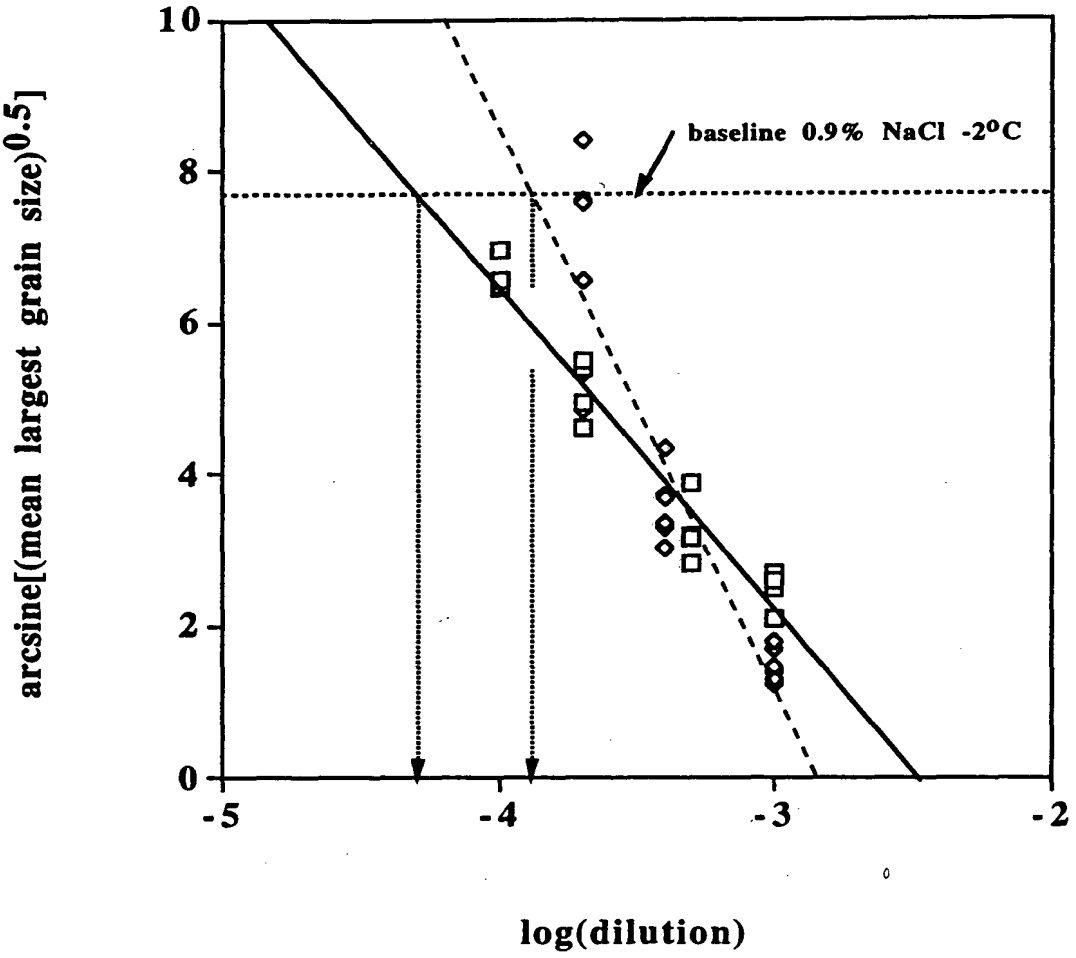


Fig. 8.29

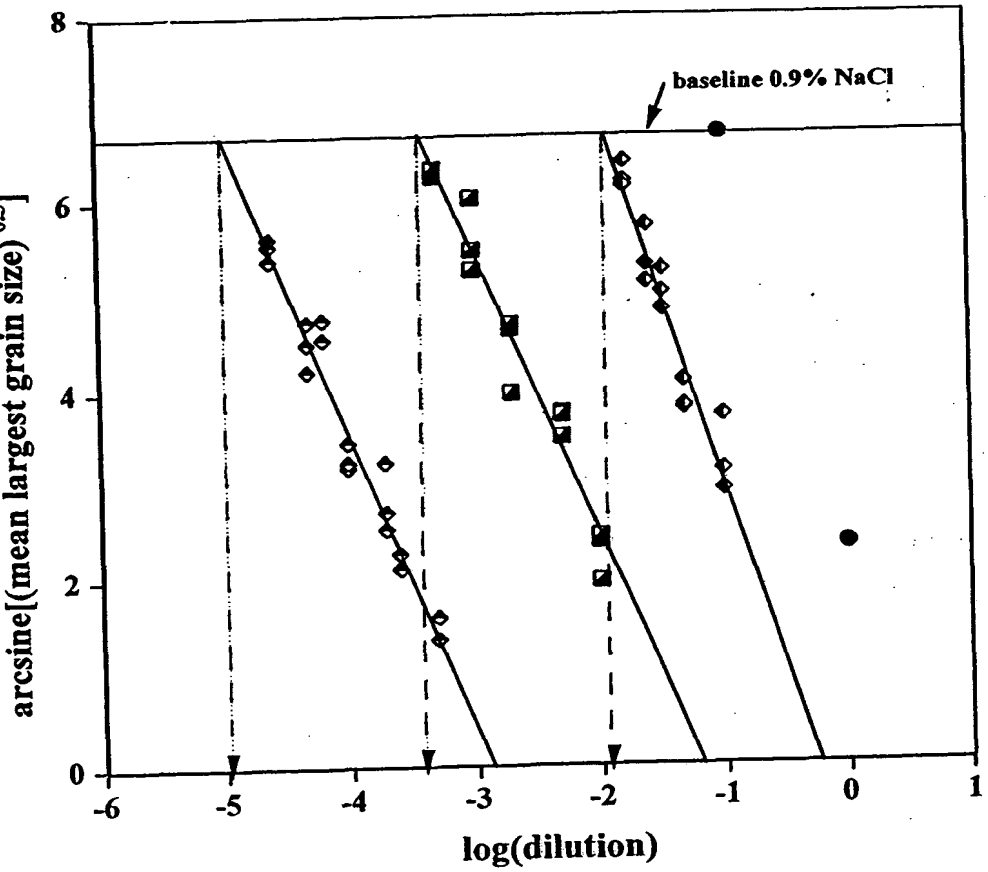

baseline 0.9% NaCl
arcsine[(mean largest grain size) 0.5]
log(dilution)
8
6
4
2
0
-6
-5
-4
-3
-2
-1
0
1

Fig. 8.30

20120-95960

Fig. 8.31

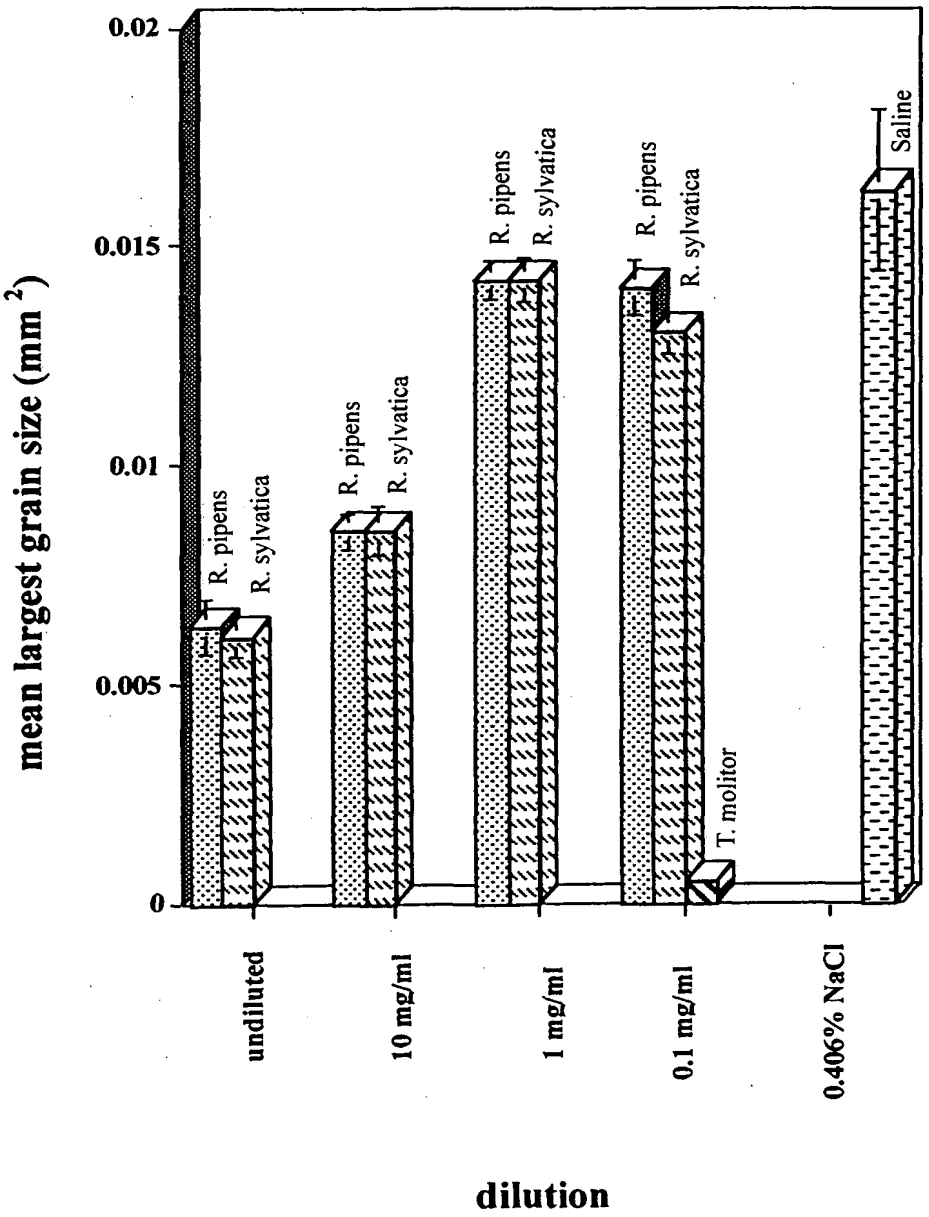

mean largest grain size (mm 2)
0.02
0.015
0.01
0.005
0
R. pipens
R. sylvatica
R. pipens
R. sylvatica
R. pipens
R. sylvatica
R. pipens
R. sylvatica
T. molitor
Saline
undiluted
10 mg/ml
1 mg/ml
0.1 mg/ml
0.406% NaCl
dilution

Fig. 8.32

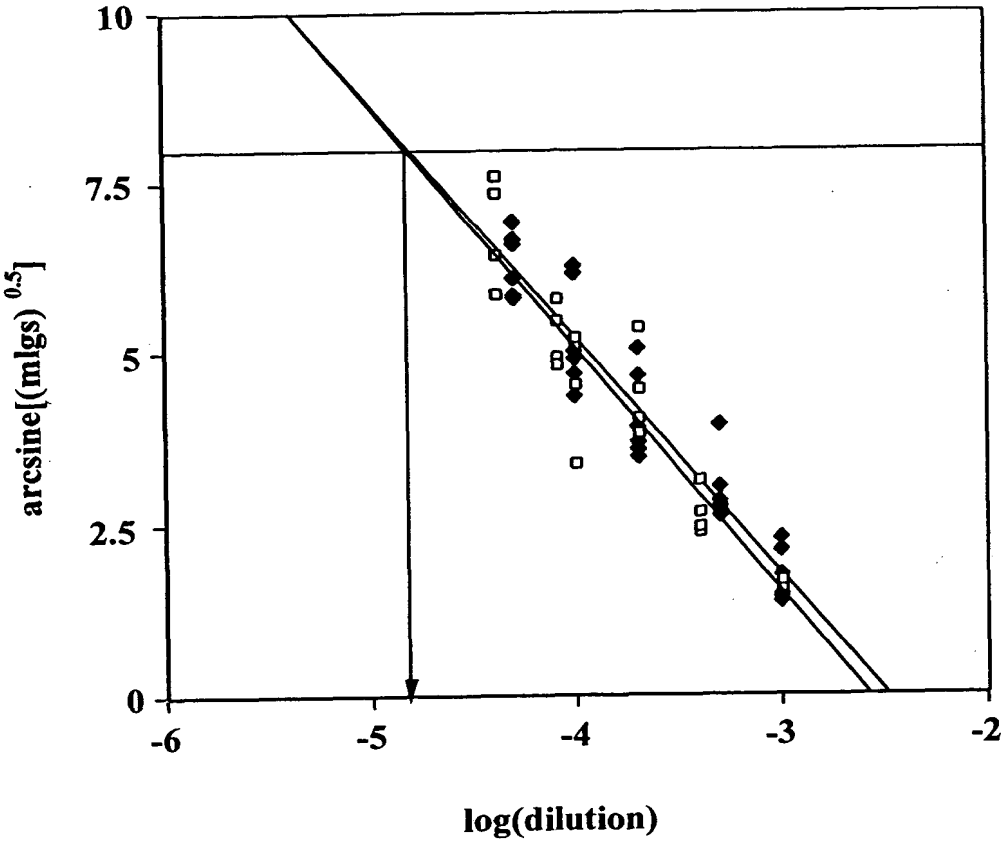


Fig. 8.33

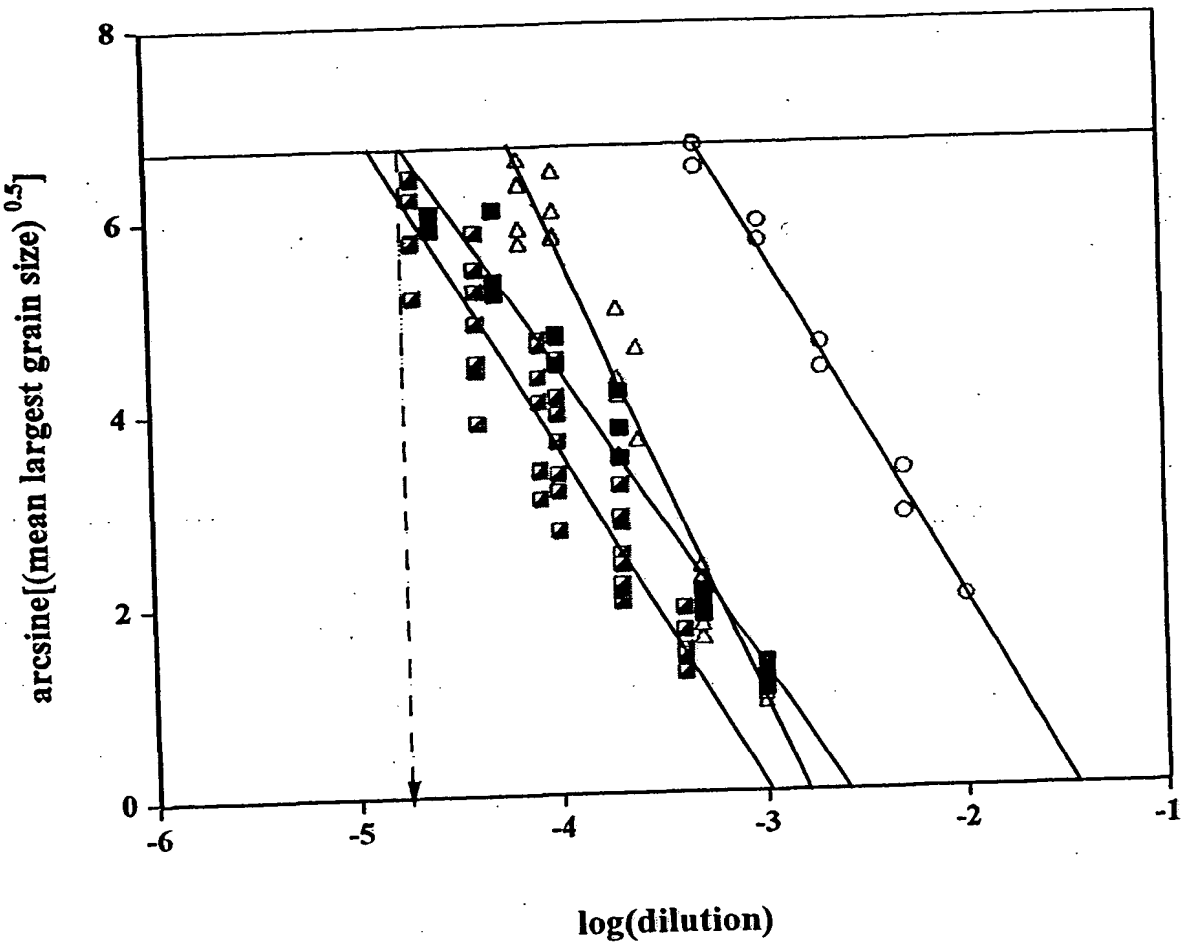

8
6
4
2
0
-6
-5
-4
-3
-2
-1
arcsine[(mean largest grain size) 0.5]
log(dilution)

Fig. 8.34

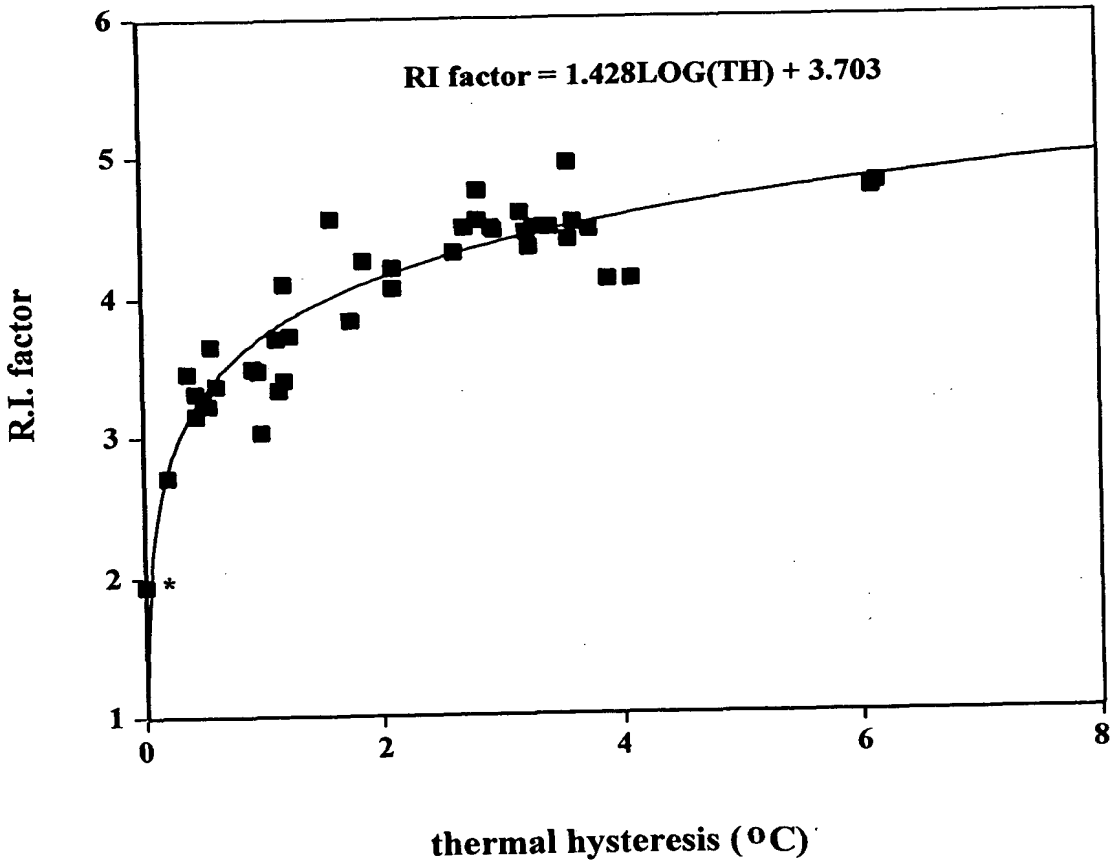

RI factor = 1.428LOG(TH) + 3.703
R.I. factor
thermal hysteresis (°C)
6
5
4
3
2
1
0
2
4
6
8
*

Fig. 8.35

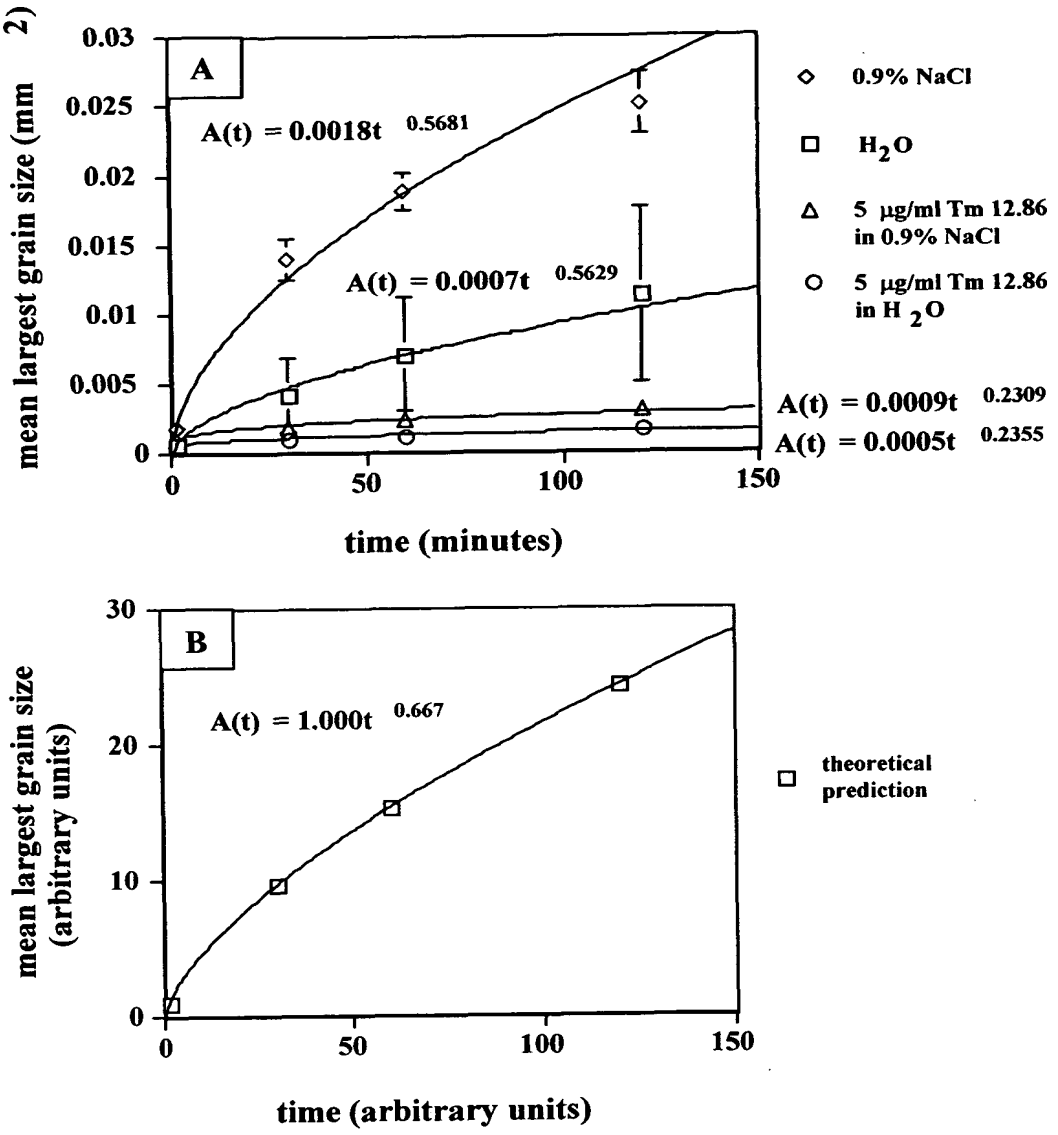
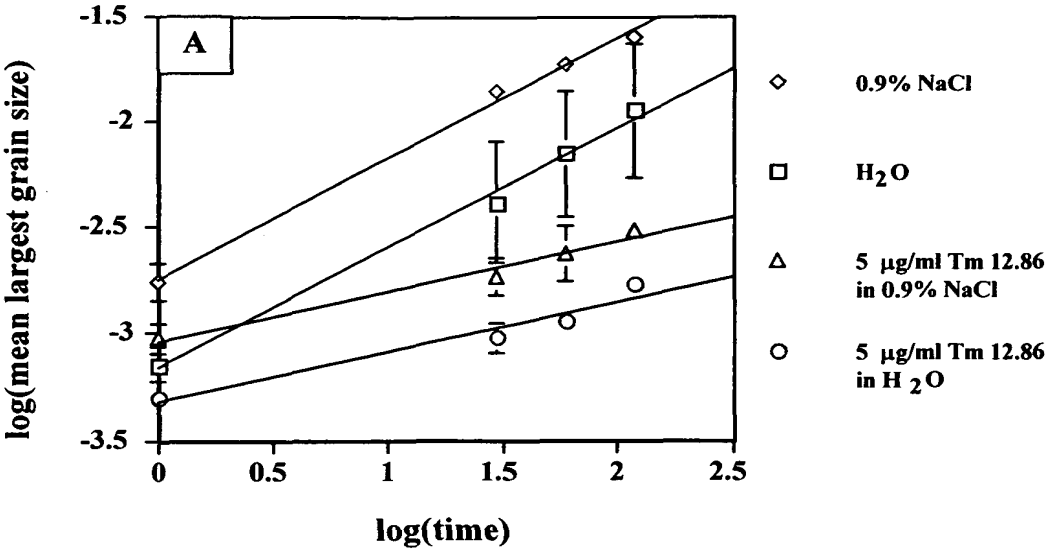

A
mean largest grain size (mm 2)
0.03
0.025
0.02
0.015
0.01
0.005
0
0
50
100
150
time (minutes)
A(t) = 0.0018t 0.5681
A(t) = 0.0007t 0.5629
A(t) = 0.0009t 0.2309
A(t) = 0.0005t 0.2355
0.9% NaCl
H2O
5 µg/ml Tm 12.86 in 0.9% NaCl
5 µg/ml Tm 12.86 in H 2O
B
mean largest grain size (arbitrary units)
30
20
10
0
0
50
100
150
time (arbitrary units)
A(t) = 1.000t 0.667
theoretical prediction

Fig. 8.36


A
log(mean largest grain size)
-1.5
-2
-2.5
-3
-3.5
0
0.5
1
1.5
2
2.5
log(time)
0.9% NaCl
H2O
5 µg/ml Tm 12.86 in 0.9% NaCl
5 µg/ml Tm 12.86 in H2O

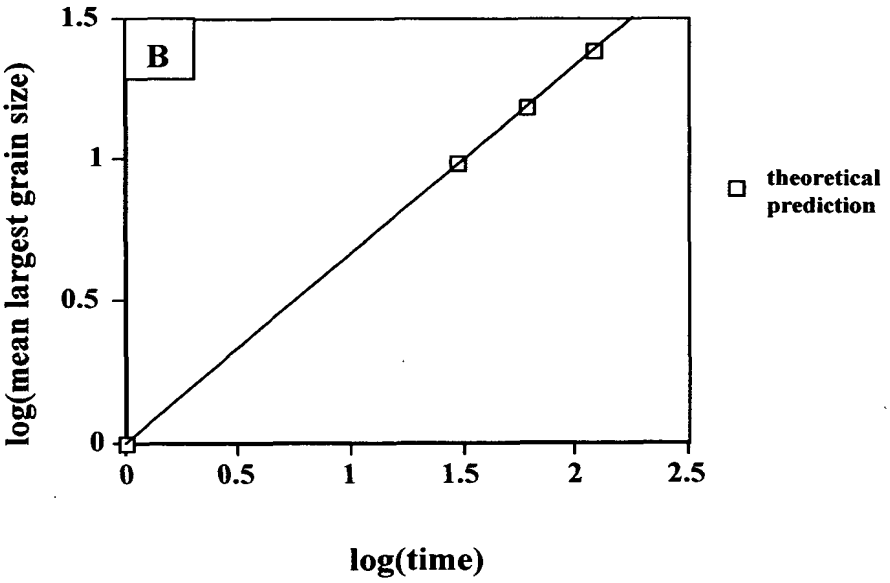
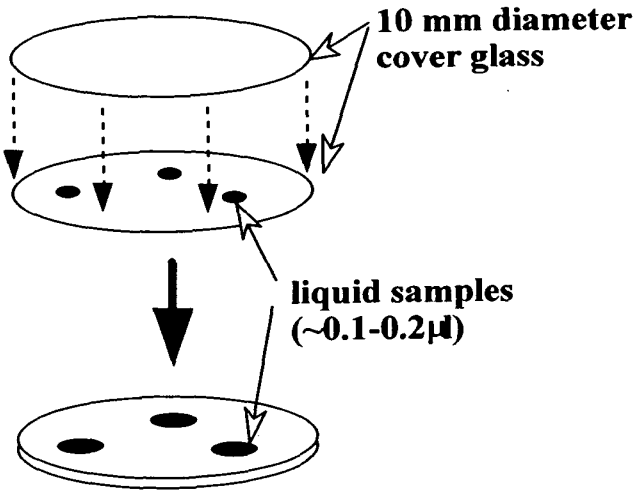

B
log(mean largest grain size)
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
log(time)
theoretical prediction

Fig. 8.37

"Sandwich" method of R.I. assessment

1.



2.

3. FREEZE ON ~-80 C
ALUMINUM PLATE (~10 MIN.)

4. PLACE ON COLD STAGE,
ANNEAL AT -6.C UP TO
12+ HOURS

Fig. 8.38

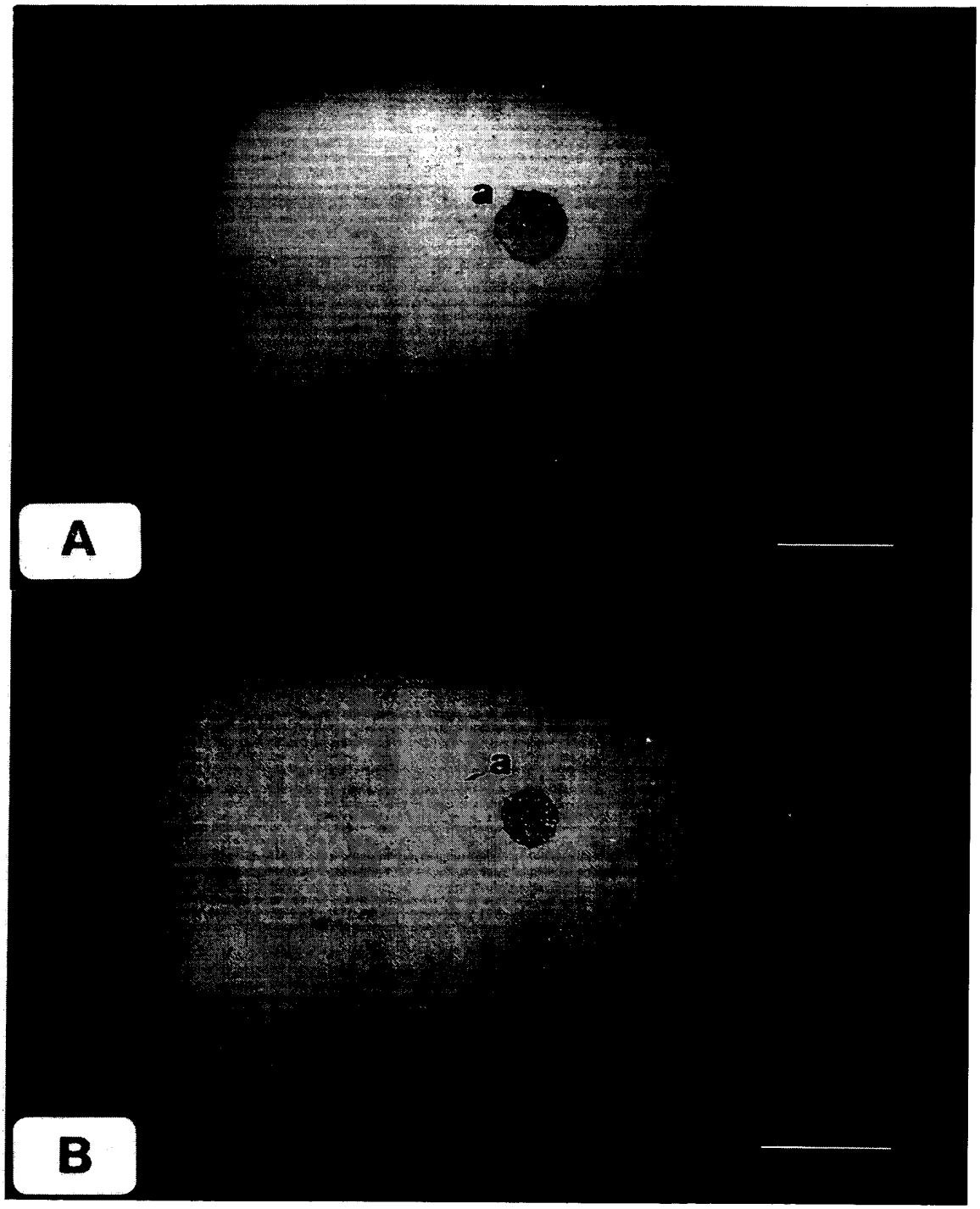


Fig. 8.39

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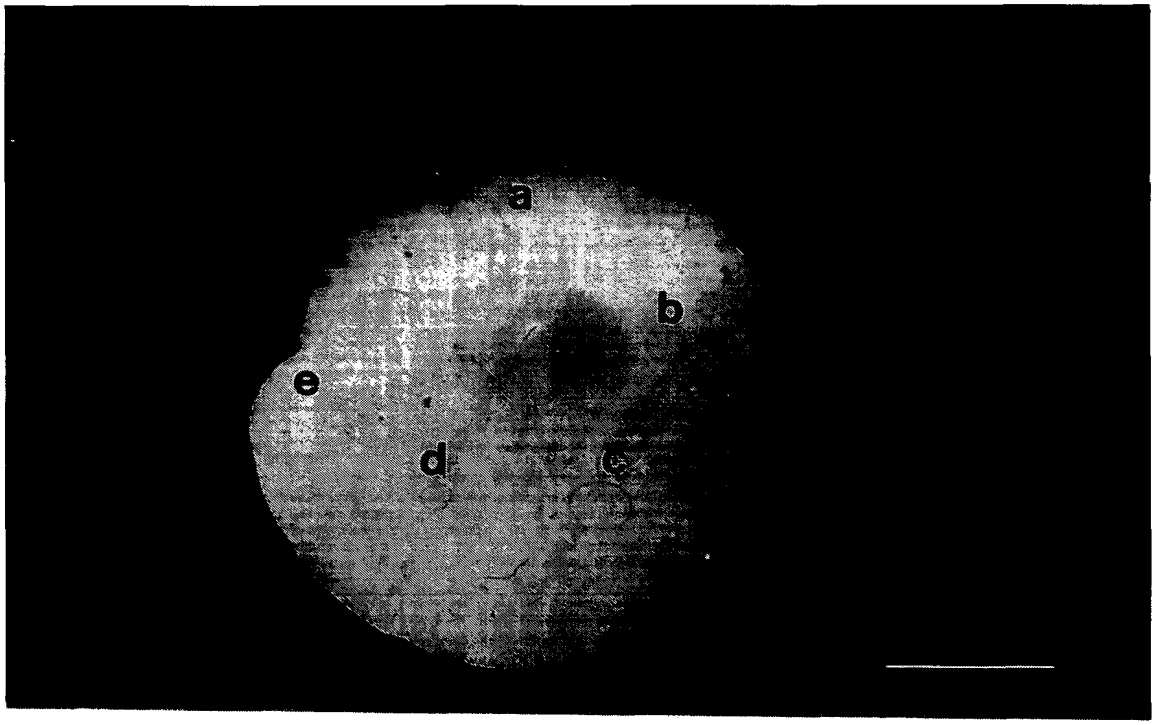

Fig. 8.40

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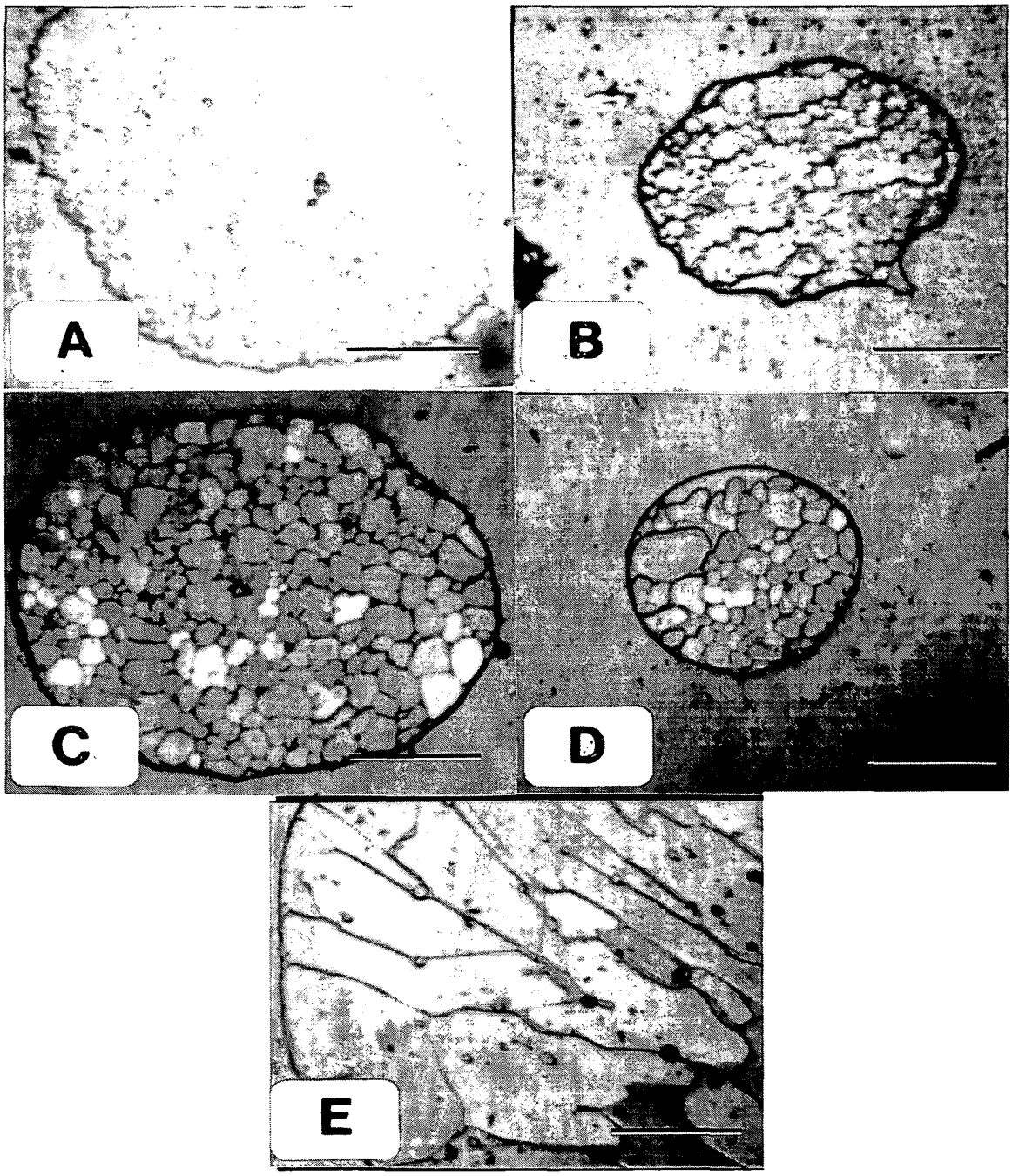

Fig. 8.41

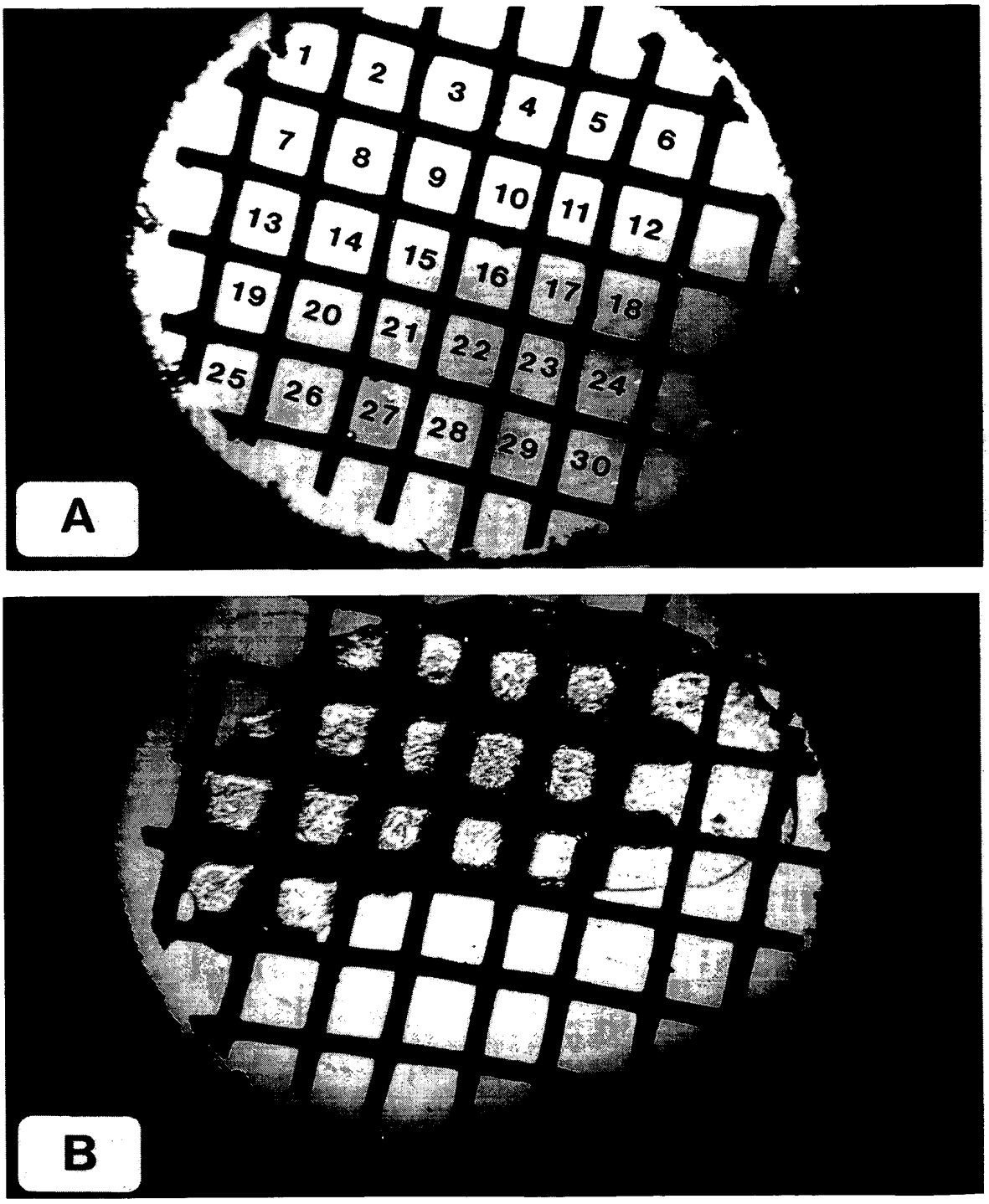
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
A
B

Fig. 8.42

DNA sequence of Tm 13.17 cDNA clone

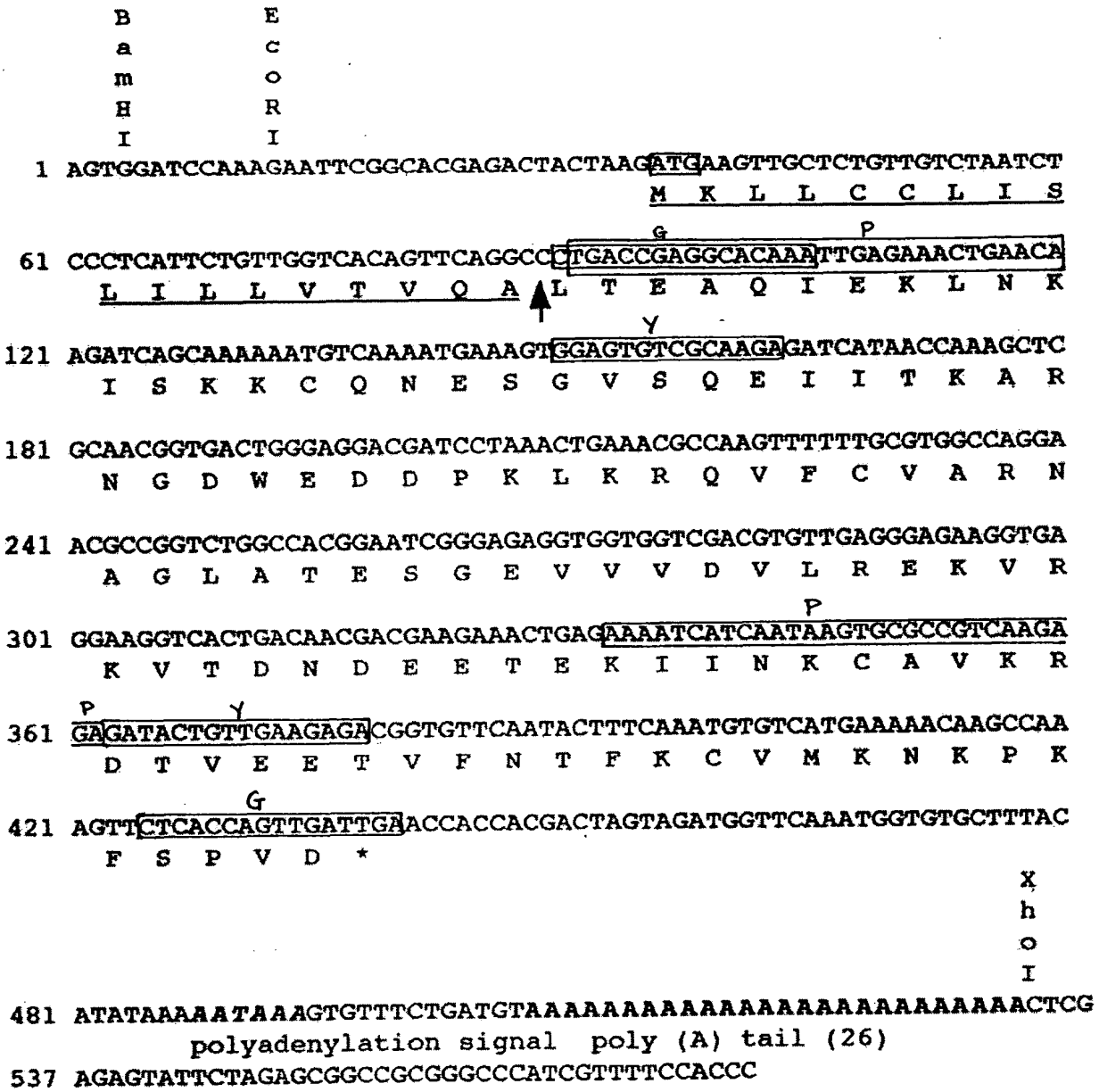

Fig. 8.43